ESSENTIAL HYPERTENSION

AN INTERNATIONAL SYMPOSIUM

BERNE, JUNE 7th 10th 1960
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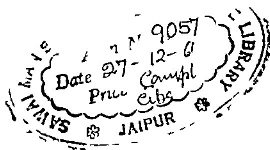
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Opening remarks

By

F C Reubi

It is a great honour for me to open this symposium and I should like to extend to you a most cordial welcome and to thank you for accepting our invitation I am sure you will all wish me on your behalf to express our sincere gratitude to Ciba Basle for financing this undertaking

Although the question of arterial hypertension has commanded the attention of research workers ever since its existence was first discovered we still seem to be a very long way from having solved the problem On the other hand while it was once true to say that medical research was confined to only a few countries we can derive satisfaction from the knowledge that today a great deal of work has been accomplished during the past few years in both the old and the new world in the East and in the West For this very reason it is becoming more and more important to ensure that results should be compared opinions exchanged and trends of research defined periodically on an international plane The more widely differing the concepts presented the more fruitful we can expect the resultant clash of ideas to prove As men of science we have no need to concern ourselves with the battles being waged in the field of international politics Let us therefore ignore such matters and seek to reconcile our views here in a spirit of understanding and open mindedness Let us banish all preconceived notions and beware of allowing ourselves to be blinded by questions of dogma prestige and personal pride

Attending this symposium are some forty specialists from 12 different countries The number of participants has deliberately been limited and the discussions will be held in private You will all know yourselves from experience that an atmosphere conducive to a frank exchange of views — views that are sometimes diametrically opposed — can only be established if the speakers cut themselves off from the outside world We have however made allowance on the programme for two sessions in public In today s public session we shall bear three lectures in which the problem of the pathogenesis of hypertension will be dealt with from the general

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The mosaic theory of hypertension

By

I H PAGE

It has been some years now since I proposed the mosaic theory of hypertension and it is time I think to review where it stands today Has it proved useful and is it likely to continue to do so?

The mosaic theory had its background in the fact that thirty years ago thinking in the field of infectious disease mostly prevailed not only in its own field but in others as well The search was often limited to the finding of a single causative agent If the typhoid bacillus was isolated and cultured this was the cause of the disease and its elimination was the treatment So those of us who worked in other fields tried to find *the* cause of hypertension and *the* cause of atherosclerosis As a result of repeated failures as many theories sprang up as there were investigators The chief contender for the cause of hypertension in those days was the kidney Many felt that after GOLDBLATT work the kidney was the true and only cause of essential hypertension The fate of this view is an interesting story but it is diverting

As time passed it slowly became evident that the problems of cardiovascular disease and cancer were not likely to yield to an attack on one front It seemed unlikely that all types of hypertension and arteriosclerosis would be explained by single mechanisms And it seems to me that reflection on the nature of the circulation gives a clue as to why this is so Arterial blood pressure is one of the components of the system used to perfuse tissues with blood The problem of getting the right amount of blood to the right part of the body at the right time is an amazingly complicated and difficult one To know where blood is available and from where it can safely be withdrawn is a major problem in itself It is not surprising then that the body has developed a highly complex system for carrying out this task efficiently We shall again put this wonderful mechanism to the test when gravitation has become but a memory in our exploration of outer space It is my guess that man is more likely to have trouble with his cardiovascular responses to weightlessness than with his control over his actions by his brain which is certainly a change

[1] Poslutn Essential Hypertension

aspect The second public session — to be held at the end of the week in connection with the Annual Meeting of the Swiss Society for Internal Medicine — will take the form of a panel discussion on the treatment of hypertension The symposium itself will be entirely devoted to the study of two specific questions the first being the problem of the possible relationships between so called essential hypertension and salt and water metabolism and the second the *long term effects of anti hypertensive therapy as regards* the clinical course of hypertensive disease We are convinced that rather than attempt to cover the whole subject of essential hypertension it is a sound procedure to concentrate our attention on certain particular aspects of this vast problem Perhaps this approach will enable us to get closer to our objective and finally to reach agreement on a certain number of points — in which case we shall be in a better position to offer some practical recommendations when we come to our second public session

You may possibly wonder why we chose these two topics for discussion in preference to others As regards the connections between *hypertension and salt and water metabolism we have the* impression that this problem has become of great current interest since the salt diuretics were introduced in the treatment of hyper tension and that the whole question deserves to be reconsidered in the light of recent endocrinological findings As for the long term effects of purely hypotensive therapy on the course of the disease the time that has elapsed since effective anti hypertensive agents were first introduced should now be sufficient to enable us to make a preliminary assessment of the value of such treatment Since this is a matter of major practical importance we should certainly not wait too long before undertaking a critical analysis of the results obtained to date

Let us hope that this symposium will prove of great help in enriching our knowledge

inant on one patient it does not mean that the endocrine cardio vascular and renal systems have quit their participation It is just that they participate to a lesser degree

Primitive and sophisticated control

I like to think of the circulation as having two levels of operation the one primitive and largely chemically controlled the other a more highly integrated one and nervously controlled The prolonged slow changes in pressure and/or perfusion may be chiefly set in motion by humoral mechanisms and the fine quick changes by the autonomic and in part voluntary nervous system In fact the two systems must be closely integrated themselves to be the effective unit they actually constitute

Even within the humoral and neurogenic mechanisms themselves there are degrees of primitiveness For example I have suggested that serotonin is in some ways a primitive norepinephrine In invertebrates it seems to be able to act as a neurotransmitter a function largely replaced by norepinephrine in vertebrates The endocrine secretory function of some neurones in insects is an example of the more primitive function of the nerve cell which may not be as highly developed in man Yet there may well be important functional vestiges in higher animals which have so far been overlooked I think until very recently we were inclined to relegate the pineal body to oblivion Whatever its function it is quite apparent that things of importance transpire within its orbit I have often suspected that the pressor material cerebrotonin which TAYLOR and I (1) showed coming from the stimulated brain might have represented endocrine secretion of neurones Of course this is just a guess which is at least better than a bad experiment

Arterial blood pressure and tissue perfusion

Arterial blood pressure is only one of the components of the mechanism of tissue perfusion But before I turn to consideration of some of these varied facets I would like to mention the discussion going on between those who believe that the increase in peripheral resistance which occurs in hypertension is due chiefly to humoral and neurogenic mechanisms and those who look upon it as principally anatomical and biophysical due to the swelling and overgrowth of the arteriolar wall or to the inherent physical properties of vascular smooth muscle Probably all are involved The question rather is how much of each

I have suggested in the mosaic theory that the varied facets which compose blood pressure control are in equilibrium with one another and the final pressure level is determined by the equilibrium point Thus if blood volume changes neurogenic vasomotor tone changes to keep the blood pressure at a constant level While one facet may temporarily play a dominant role in determining the level this does not mean that all the other facets cease functioning Just so in the hypertensive patient one facet may be dominant but the secondary facets are in equilibrium with it and, with the passage of time may themselves become primary

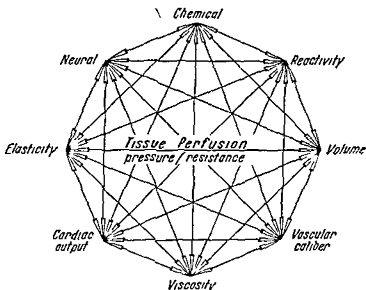


Fig 1 The mosaic theory of hypertension showing the equilibration of the various facets of pressure control to provide for controlled tissue perfusion

This seems a simple concept indeed with which no one should quarrel but few physicians truly understand it For instance it explains why there are so many possible points at which the *mechanisms of blood pressure control* can be blocked for therapeutic reasons It explains why the clinical picture of hypertension is so variable Not the least of its virtues is that it makes thinking about the disease orderly

Beyond the physiological processes which I have included in the octagon I have further divided and grouped what seem to be primary mechanisms of a variety of hypertensions These groups are 1 nervous 2 endocrine 3 cardiovascular and 4 renal Again it must be recognized that while the nervous system may be predom

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We assume that if the sympathetic nerves are cut this removes all tonic vasomotor impulses What vascular tone remains must then be due to blood borne vasoconstrictors or to the anatomy of the blood vessels *It has been further assumed that* complete relaxation of the blood vessels may be achieved with such drugs as acetylcholine ATP or nitrites The degree to which various *vascular areas are under sympathetic control* varies enormously as Uflander and Folkow (2) have shown The relative importance of the composition of the bathing medium for the tone of denervated *blood vessels has not, so far as I know* been adequately studied In this composition I would include both the usual constituents of the blood and any humoral agents that may be secreted into it *Such an* experimental demonstration is difficult because of the subtlety of the possible compositional changes as well as the length of time required for such changes to be reflected in the tone of the blood vessels

From the days of Cohnheim and Gull and Sutton the notion has existed that thickening of the blood vessel wall preceded the hypertension and was the cause of it The arguments for and against this concept are too well known to this audience to waste their time repeating them Some elaboration has recently occurred in that thickening as a result of electrolyte changes in *the vessel* wall has been added to thickening due to hypertrophy and hyperplasia

One more factor concerned with peripheral resistance is inherent in the vascular wall itself Automaticity or vasomotion of the smooth muscle is considered one of these factors The distending force of the *blood* pressure creates *in smooth muscle a* tendency to rebound and thereby induces some degree of vascular tone

Applying this *type of thinking to the hypertensive is no more* satisfying than it is in the normotensive Theoretically it is clearly of importance but so far little objective measurement of the *separate components of this complex mechanism of maintenance* of vascular tone is possible in patients or indeed in intact animals

Whether vascular resistance when smooth muscle is completely *relaxed is the same in hypertensives as in* normotensives would depend at least on the stage of the disease Early it may well be the same but with time development of vascular disease could *scarcely* help increasing it The impressive thing to me is the extensive anatomical change that can occur in hypertensives and yet maintain their ability to vasodilate Folkow (3) stresses the fact that it takes only a small decrease in the internal diameter of the

maximally dilated blood vessel to cause a pronounced effect on the resistance to flow a five percent decrease causing the resistance to be raised something of the order of 25 % Further it can be calculated that for any given shortening of the smooth muscle the resistance of a hypertrophied vessel would be increased proportionately more than in normal vessels because a larger tissue volume intrudes on the vascular lumen At the same degree of smooth muscle tension the hypertrophied vessel for purely mechanical reasons exhibits a greater vasoconstrictor action than normal (4)

FOLKOW GRIMBY and THULESIUS (5) tested this hypothesis by measuring forearm blood flow in normo and hypertensive subjects in whom an attempt was made to elicit maximum vasodilatation by ischemia and work Dilatation was further facilitated by heat If in fact these measures yield maximal dilatation then in some well established essential hypertensives resistance remained moderately raised They point out that there are serious technical difficulties with this method and that the results can only be looked upon as suggestive

Experience with the antihypertensive drugs has done much to dispel a firmly fixed notion as to the so-called fixed hypertensive It is relatively *rare* in my experience to see a patient in whom the supine blood pressure cannot be reduced *to normal* and when it is normal to exhibit symptoms or signs of ischemia This is not *proof* that some residue of structural change in the resistance vessels is not still present but it indicates that stronger proof of its paramount importance must be forthcoming I would like to venture the opinion that increases *in peripheral* resistance as a result of structural vascular change may be important in certain specific areas such as the renal myocardial and *cerebral* vessels but that a generalized increase in resistance due to structural change is less significant comes late in the course of the disease and is commonly to some degree reversible

Distribution of blood according to local need

Surely it is the need of organs which determines the distribution of blood Organs undoubtedly can *store* blood in amounts above their need but lack of blood is a condition essential organs *do not* tolerate I say *essential* because it is well recognized that some vascular areas may be almost closed down during periods of great need in other areas But when the heart the brain some endocrine glands and the kidneys need blood they usually get it

The mechanism by which this is accomplished must be more than mere passive dilatation from local axon reflexes or locally formed metabolites allowing more blood to drain into the dilated area I should suppose that the need is signaled by neurones and humoral messages indicating need and that after integration the appropriate afferent messages go to those vascular areas where blood can be spared

In keeping with what I have said before for quick changes in blood distribution I would expect nerve mechanisms to be chiefly involved but for prolonged changes humoral mechanisms Are there then such physiological mechanisms demonstrable?

The kidneys demonstrate the humoral mechanisms admirably though I must admit that the demonstration is not rigorously proved (58) I shall not discuss the mechanism at this point but simply say that when the kidneys do not receive enough blood or there is a change in the pulsatile character of the blood received a reaction is set up which raises blood pressure which may aid in overcoming blood deficit The nerve bundles supplying the kidneys have always been a puzzle and like those supplying the brain seem to have only mild vasoconstrictor action and do not appear to take an important part in circulatory control except possibly under conditions of great stress McCUBBIN and I (6) found some evidence of nervous connections with the adrenal glands which could be stimulated by the ganglion stimulating agent Dmpp but the actual function of such connections is purely conjectural

The brain in respect to its control of its circulation is a good deal like the kidneys It is highly dependent on the height of the systemic blood pressure partially on humoral agents and with relatively little dependence on vasomotor control On the other hand vasoconstriction and dilatation within the brain can importantly influence vasomotor reflexes such as the reflex pressor response to occlusion of the common carotid artery KANEKO McCUBBIN and I (7) found that several vasoconstrictor drugs given into the cerebral lateral ventricles as well as cooling of the cerebrospinal fluid inhibited this reflex The effect was opposed by central administration of vasodilator drugs or heating the cerebrospinal fluid Vasoconstriction in the cerebral vessel was associated with a fall in systemic arterial pressure and slowing of the heart rate while local vasodilatation opposed these central inhibitory effects I shall discuss this phenomenon later but I want to use it now to illustrate the thesis that the blood flow to an organ can influence systemic blood pressure to an important degree The brain unlike the kidney seems to exert its influence largely on the cardio

vascular system by changes in neural control But even the brain seems to have some modicum of direct chemical control if the pressor substance tentatively called cerebrotonin can be more closely identified and characterized At one stage in its evolution the brain exhibited relatively highly developed endocrine function which may not have been wholly lost

The control of blood to the myocardium seems to combine the neural and humoral mechanisms to give each clear importance When the heart lacks blood it cries out for more during the familiar angina pectoris Its afferent control is highly responsive The efferent control affects both inotropic and chronotropic characteristics of the muscle but it is less clear how effective the vasomotor response may be The same may be said for the control by the blood borne vasoactive agents although again it is clear that both have important effects even though their quantitative relationships are not defined We know too little of the meaning of the storage of catecholamines and other vasoactive agents in the myocardium and blood vessels to speculate profitably

Each organ could be considered in similar fashion but enough has been discussed I believe to illustrate the thesis that the local need for blood is one of the critical determinants of systemic blood pressure From this it follows that disturbances in the level of blood pressure may result from disturbances in the need for blood in single and multiple organs The dominant mechanism employed by an organ to control its own perfusion may under some circumstances assume a primary role in controlling systemic blood pressure levels However the dominance of one mechanism does not insure the abrogation of all other controlling mechanisms It is for this reason that arterial blood pressure may be affected by agents acting on such different facets of the blood pressure controlling mechanism

1 Neurogenic participation

Clinical	*Experimental*
Poliomyelitis of brain stem	Cerebral ischemia
Porphyria chronic	
Increased intracranial pressure	Cushing s experiment
Sclerosis of carotid sinus	Resection of sinus and aortic depressor nerves
Resection of glossopharyngeal nerve	
Emotion	Hypertension from audiogenic stimulus

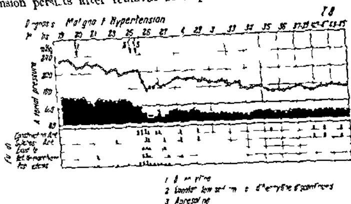
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For example it may explain why nephrectomy lowers pressure in the acute stage of renal hypertension but not in the chronic It might be a factor in the occasional patient in whom the hypertension persists after removal of a pheochromocytoma and in



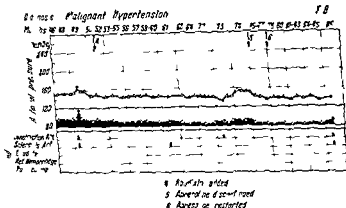


Fig Patient with malignant hypertension treat d su c ssfully with hydral lne f r y ars W? the drug was w thdrawn blood pr ssure did not rise Blood press res as d pl y d by d is represe t e re of 4 m asurem nts a day f r o week Triangles represe t a erage tak t hom ur s patient line Eyegrou d are grad d on a 1—4 scal Failure of th ympath tic blocking g nt Diben ylin to l wer arterial pressure is hown at arrow 1

the group of patients we have found to become normotensive after years of antihypertensive therapy An example of such a patient is shown in Fig 2

The carotid sinus mechanism is only one of several nervous mechanisms which can control blood pressure in a discriminating fashion Much work has shown the highly selective action of

As I promised I shall touch only superficially on a few topics of special interest to illustrate my thesis there will be no marshalling of evidence to show the important participation of the nervous system in the mechanisms of hypertension We have already done this in outline form recently (*59*) I shall first consider the problem of the resetting of the carotid sinus and similar buffer mechanisms when renal hypertension develops *merely as an* example of the behavior of the nervous system under the changed conditions of hypertension

Blood pressure regulatory mechanisms and the level of their "set"

Physicians have been puzzled for many years because of the fact that pressure on the carotid sinus area elicited the same circulatory response in hypertensive subjects as in normotensive *ones It had been taught that the sinus mechanism was one of the* most powerful modulators of blood pressure levels When blood pressure is raised by injection of pressor drugs baroceptors are *stimulated and reflexly diminish or eliminate neurogenic vaso* constriction from the vasomotor centers in an effort to lower blood pressure/But when the pressure rises during the course of essential *or malignant hypertension the sinus mechanism seems to fail* Indeed it seems to operate to maintain the elevated pressure

McCUBBIN GREEN and I (*8*) studied this problem using electro*neurographic techniques to measure baroceptor activity in normo*tensive states and then after production of acute and chronic renal hypertension The observation that in chronically hypertensive *dogs baroceptors fire intermittently at supernormal pressure* levels — which elicits continuous firing in normotensive animals — indicates that the regulating mechanism has been set to buffer *arterial pressure at higher levels* 'The carotid sinus buffer mechanism has shifted its threshold and range of response upward so that it perceives so to speak the hypertensive levels as normal ones The regulator has been reset upwards After the resetting it naturally acts to maintain arterial pressure at the higher level rather than normal ones

Clearly even hypertension of primarily renal origin has an important neurogenic component and it is in my opinion the reason that patients with such hypertension respond to neuroplegic drugs in many cases as well as patients with essential hypertension in whom the renal component is less clearly defined The resetting phenomenon can be used as an explanation for many of the pharmacological and clinical phenomena associated with hypertension

system Reserpine is an excellent example It is known to have a strong central cardiovascular action but the mechanism is not known Recently Drs McCubbin Kaneko and I (9) studied this problem in dogs Both serotonin and norepinephrine are released from the bound to free form in the brain by reserpine Both inhibit central synaptic transmission It is therefore possible that the central effects of reserpine on vasomotor activity depend upon release of these two amines and it should be possible to reproduce these effects by injecting them directly into the cerebrospinal fluid since reserpine is effective when given in this way We tested both serotonin and norepinephrine and their respective precursors

All of these substances had qualitatively the same effects in that they lowered arterial pressure usually caused bradycardia despite prior vagus nerve section and caused marked inhibition of the pressor response to occlusion of the common carotid arteries The same result was obtained in anesthetized and unanesthetized dogs These results are all consistent with the premise that the acute cardiovascular effects of reserpine are mediated centrally by serotonin and/or norepinephrine either released from a bound and inactive to a free and active form or formed by decarboxylation of their respective amino acids

But how might they do this? We found that not only reserpine but several vasoconstrictor drugs given into the cerebral ventricles *inhibited the carotid* occlusion pressor reflex This effect was opposed by central administration of vasodilator drugs Cooling of the cerebrospinal fluid which presumably caused local vasoconstriction also caused inhibition of the carotid reflex hypotension and bradycardia — effects *counteracted by* central injection of vasodilator drugs Warming the cerebrospinal fluid did just the opposite It would *appear* then that these effects on vasomotor activity depend upon changes in local blood flow It follows that the cardiovascular effects of reserpine are probably due to local decrease of tissue perfusion caused by release of serotonin norepinephrine or other vasoconstrictor agents

From this and the example taken from the resetting phenomenon in the carotid sinus mechanism one begins to feel the power and complexity of the nervous *control of* arterial blood pressure and tissue perfusion

Cardiovascular participation

Clinical	*Experimental*
Coarctation of aorta	Clamping of aorta above renal vessels
Heart failure	
Arteriovenous fistula	
Arteriosclerosis	

concentration of circulating red blood cells and plasma proteins) diastolic hypertension is inevitable Hypertension, then, can be considered a form of generalized vasospasm, a variant of normal *responses to circulatory stresses*, characterized by healthy blood and heart and by its chronic nature Any one of several of the mechanisms which produce vasospasm can be involved in causing hypertension, new mechanisms can also arise at a time long after the organism would be dead or recovered from shock, had that been the initiating cause

While we do not know the exact causes of generalized vasospasm, we do know a great deal about the mechanisms which produce it The causes themselves lie in biochemical alterations in blood vessels nervous tissue and organs of high metabolic activity It is not enough to say that we do not know and therefore we must not try to know For there are not many areas which have been so well studied, and there is little need to invoke esoteric mechanisms to the neglect of known ones If one asked any physiologist what influences he would think could raise diastolic blood pressure on the basis of experimental data he would choose autonomic nerves, kidneys and endocrine organs He would not choose liver, thyroid spleen or pancreas, however, but consider sympathetic nerves and their origins, renal blood flow and adrenal glands

Clinical Chronic Hypertension We can transfer these thoughts of our physiologist immediately to our own clinical experiences viewing each case in their light Let us take some typical ones

1 A woman of 25 was found on routine examination to have a blood pressure of 152/88 mm Hg which after a rest fell to 138/82 Her mother had hypertension without sequelae She had no symptoms, but her physician noticed that her heart rate was a little rapid and that she

at 56 At autopsy were found cerebral hemorrhage massive arteriosclerosis moderate of aorta renal arteries coronary arteries marked of circle of Willis with rupture arteriolar nephrosclerosis moderate cardiac hypertrophy (460 Gm) Her adrenal glands were normal

This sequence of events leading to early death can be reconstructed in the light of what is known For many years this woman reacted to stressful situations by neurogenic vasospasm Slowly the reversibility of this alteration became less and less some gradually increasing factor being added which maintained a basal blood pressure at higher and higher levels upon which was engrafted a widely fluctuating neurogenic component This added factor was what killed her Perhaps she would not have died so early were it not for another disease atherosclerosis which began probably after her menopause and affected her cerebral arteries to such an extent that one ruptured under the high pressure

2 A man of 25 was found to have a slightly elevated blood pressure and tachycardia when examined for the *draft*. His mother was hypertensive and his father had died of a coronary occlusion at the age of 51 Enuresis until the age of 8 had occurred but he was free of further *urinary* symptoms and his urine showed no albumin He went to his family doctor who found a few bacteria in his urine with about 10 white blood cells per high power field in the centrifuged sediment repeated cultures showed non hemolytic streptococcus of the colon group in large numbers He was given phenobarbital and was accepted for duty in the Army He had a creditable career and won several decorations for bravery His discharge physical examination showed a blood pressure of 160/100 mm Not until the age of 35 did he consult a physician for severe headaches and blurring of vision which had appeared a month earlier His blood pressure was 236/160

every 4 hours, it was normal during sleep (140/90) and dropped to 122/88 when tetra-ethyl ammonium chloride was injected intravenously She was given phenobarbital but was never the same Investigative studies on the renal clearance of para aminohippurate and inulin revealed re duction of renal plasma flow and relatively increased glomerular filtration rate the profile of efferent arteriolar spasm

Emotionally induced vasospasm had added to it another factor In the first place the neurogenic influence had in creased Secondly the reversibility of the vasospasm had lessened

She had her menopause at 45 By the age of 48, she was suffering from headaches every morning anxiety in creased nervous tension and an inner sense of excitement Her blood pressure was now constantly over 200 mm systolic in spite of sedatives but fell with rest to 160/110 fluctuating widely During sleep induced by heavy seda *tion it did not fall to normal* Her fundi now showed some sclerotic tortuous arteries her electrocardiogram indi cated enlargement of the left ventricle seen also in x ray photographs Occasionally she had a trace of albumin in her urine but she excreted 30 per cent of intravenously injected phenol red (PSP) in 15 minutes and her kidneys were able to concentrate urine to a specific gravity of 1 025 The blushing became more pronounced

Now had appeared an irreversible component to the vasospasm, in that sleep did not completely abolish it Signs of organic damage were developing Rest reassur ance sedatives, superficial psychotherapy and laying on of hands could not interrupt the vicious cycle

She suffered her first stroke of apoplexy a mild one at 51 and recovered with little residual other than a limp Hypertension persisted unabated and she died of another

kidney and cardiac hypertrophy (520 Gm) and dilatation His brain was edematous his adrenals normal

This man suffered from the accelerated phase ' or what is more exactly and descriptively called malignant hypertension and died young He had the constitutional make up of the hypertensive person to which was added chronic low-grade smouldering pyelonephritis with an organism which does not produce pus but causes scar tissue These two factors operating together shortened his life By the time he died there was little evidence left of the primary renal disease in the kidney distorted by nephrosclerosis

3 An older man had a slightly elevated blood pressure at times of stress which had been normal on regular examinations all of his life At 49 however he was refused life insurance because of a blood pressure of 170/110 mm Hg He had no symptoms except nervousness but he was a tense dynamic individual with excessive drive and ambition somewhat of a perfectionist. Examination in hospital revealed no significant abnormalities except minimal left ventricular enlargement studies on his renal plasma flow showed slight reduction with the calculated increased renal vascular resistance on the afferent side of the glomerulus His blood pressure varied moderately but did not fall to normal levels during sleep or the injection of tetraethyl ammonium chloride Renal "function was normal He was well working hard and taking few vacations until he was suddenly seized at age 54 with a severe retrosternal pain and was admitted to hospital with an acute coronary occlusion Other than minimal cardiac enlargement a tortuous aorta and the usual signs of infarction there were no abnormalities He recovered slowly but his blood pressure normal or low during his illness became elevated again to 180/110 mm during rest and as high as 220/120 during activity In spite of rearranging his life he remained hypertensive until his second infarction at 57 from which

mm there was 3 plus proteinuria and microscopic hematuria, the ocular fundi showed early papilledema and soft cotton wool exudates but no hemorrhages, and his heart was slightly enlarged He was able to concentrate urine to a specific gravity of only 1 019 and excrete only 15 per cent of the intravenously injected dose of phenol red in 15 minutes Culture of the urine showed nonhemolytic staphylococcus, considered a contaminant but intravenous pyelography revealed blunting of one upper calyx in *the right kidney His blood pressure altered little during* deep sleep and when terta ethyl ammonium ion was injected, his diastolic pressure fell from 158 to 145 mm Hg

This man, unlike his predecessor, had rapidly reached an irreversible stage of vasospasm His blood pressure was fixed' and his course was presumed to be rapidly progressive Some organic renal component could be inferred from the earlier urinary findings and the pyelographic evidence, but this was asymptomatic His family and early history suggested that he might be one of those persons who react to stress by vasospasm, added to this ✓ component was a chronically diseased kidney

He refused admission to hospital but was examined frequently during the next year and took the new Rauwolfia drugs continuously without effect on his slowly rising blood pressure and deteriorating condition Finally he was forced to seek help because of increasing dyspnea, but by that time the nonprotein nitrogen in his blood was 132 mg per cent his diastolic pressure 160 mm Hg or more he had suffered one attack of pulmonary edema and his ocular fundi showed many hemorrhages hard exudates soft exudates and papilledema After a stormy downhill course he died of uremia complicated by congestive heart failure At autopsy there was moderate generalized arteriosclerosis advanced arteriolar nephrosclerosis with necrosis, a few depressed scars on the cortices of one

The curious thing about her obesity was its distribution over the trunk upper arms and thighs Her lower legs and arms were not obese at all There was a "buffalo hump" and her face resembled those seen after overdoses of cortisone Measurements of the sodium and chloride in her sweat showed values of 10 mEq /L. or less levels found in Cushing s syndrome. She bruised easily and her ankles had a tendency to swell in the evenings She had a distinct mustache Serum sodium was 145 chloride 101 CO_2 32 4 and potassium 2 8 mEq /L.

She did well but remained hypertensive at home as it was impossible for her to restrict her salt intake and her appetite Not until she was 45 did her first episode of congestive heart failure bring her back into hospital She died 2 years later a cardiac cripple in the interim At autopsy were found arteriolar nephrosclerosis slight to none moderate generalized arteriosclerosis marked cardiac hypertrophy and dilatation (640 Gm.) There was a 1 x 1 2 cm adenoma in the left adrenal cortex.

In her case a functioning adenoma in her adrenal cortex was affecting both her salt and fat metabolism the former influencing her hypertension Better diagnostic methods would have allowed surgical removal

These four cases are illustrative of distinct types of arterial hypertension encountered clinically In actual practice one sees wide variations in their courses and some times bizarre mixtures If the first patient had contracted glomerulonephritis in childhood or pyelonephritis during her pregnancy or had by chance had a kink in her uretero-pelvic junction due to an aberrant renal artery with stasis and infection she probably would have exhibited more severe hypertension at an earlier age If the second had not contracted pyelonephritis in childhood he might have lived to become hypertensive in his 50 s and died of heart failure or apoplexy in his 60 s If the renal arterial

he died At autopsy there was found cardiac enlargement with focal myocardial fibrosis a new infarct arteriolar nephrosclerosis slight, and generalized arteriosclerosis Careful cross sectioning of the mouths of the renal arteries revealed some encroachment of their lumina by atherosclerotic plaques

This man also probably had the constitution for hyper tension When he developed atherosclerosis, the slight narrowing of his renal arteries interfered enough with renal hemodynamics to cause a moderate hypertension The elevated pressure worsened the atherosclerotic process, which in his case was lethal because it involved his coronary arteries Hypertension itself did little direct but much indirect harm for he died before his time

4 A woman of normal weight began to gain rapidly after her second pregnancy at age 22 Within 2 years her weight increased from 110 to 190 pounds and slowly in creased thereafter At 35 she weighed 252 but on dietary restriction lost 23 pounds Her menses had always been somewhat irregular and frequent but in her 30 s periods of amenorrhea for 3 to 6 months appeared She was found to exhibit hypertension at age 31 using the large (18 cm) cuff her blood pressure was 160/102 and using the normal (12 cm) cuff 186/122 Subsequently hypertension was moderate blood pressure seldom exceeding 200/130 mm and usually being about 200/120 Fundal changes were minimal Studied in hospital there was little evidence of vascular damage other than an enlarged heart Periods of decreased urinary output followed by polyuria were noticed when her fluid balance was measured Renal plasma flow and glomerular filtration rate were normal Weight was lost very slowly on severe dietary restriction of cal ories, but restriction of salt promptly lowered blood pres sure to normal levels Her mother and one of three sisters were fat hirsute and hypertensive

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atheromata in the third had piled up enough to cause serious encroachment on the lumina, he might have entered the malignant stage, unless heart or brain gave out first If the fourth had contracted pyelonephritis as well as her tumor, her course would have been shortened Renal disease of any nature to interfere with blood flow, when combined with the "hypertensive diathesis, can increase the severity and shorten the course The fact that hypertension is absent before azotemia in approximately 30 to 40 per cent of patients with renal diseases, points up the necessity for the presence of another factor to explain pathogenesis

Pathologic Alterations Dead house pathology does not always explain physiologic and biochemical alterations in disease But it cannot be neglected There are very few anatomic changes in hypertension, but they are characteristic

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universal although we have rarely seen normal sized hearts after sustained hypertension This change is probably a work hypertrophy resulting from the increased cardiac work necessitated by the hypertension. It can be modified by associated atherosclerosis of the coronary arteries.

3 Generalized atherosclerosis is almost universal in this country although cases without it are common in China (8)

Sequence of Development of Arteriolar Nephrosclerosis This basic lesion which by its very nature can cause renal ischemia and hypertension, is a result of hypertension In other words the altered hemodynamics of hypertension can lead to a pathologic change which further alters renal and therefore peripheral hemodynamics. The evidence is clear on this point, in rats rabbits dogs and man (Chapter V) Therefore at some point in two of our cases renal vascular disease appeared after hypertension had become well established. Whether or not this secondary disease is responsible for the loss of reversibility of vasospasm is not known, but presumably it is not wholly accountable.

Comment All biological phenomena can eventually be understood in terms of physics and chemistry The remainder of this monograph is concerned largely with an examination of the biochemical alterations possibly operating to cause this disease which is so eventually fatal as a rule and which is so common to Western Civilization.

MECHANISMS OF SOME OF THE EFFECTS OF CHRONIC ARTERIAL HYPERTENSION

Degree of Peripheral Vasospasm in Chronic Hypertension The vasospasm can be very intense in chronic arterial hypertension in fact, it must be so in order to maintain the diastolic pressure at high levels. One can estimate the intensity of the vasospasm by measuring the pressure

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in an artery peripheral to complete intermittent mechanical occlusion (9 11) In man only the brachial bed offers a convenient means of doing this In Figure 1 are shown 'asystolic arterial pressure gradients' in various types of hypertension Persons with diastolic pressures from 100 to 200 mm Hg have shown asystolic brachial pressures 18

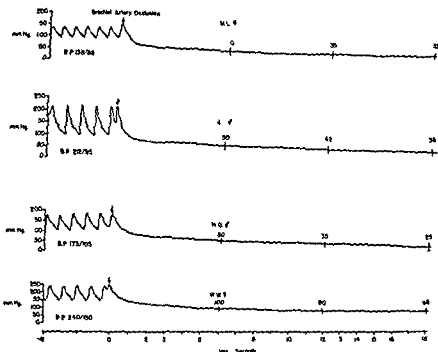


Fig 1 Brachial asystolic arterial pressure gradient in man (9 10) A needle connected to a recording manometer was inserted into the brachial artery and a sphygmomanometer cuff placed about the upper arm After rapid occlusion of the cuff the fall of pressure in the distal segment (the forearm) was measured The level of pressure with no circulation is a measure of the degree of vasoconstriction opposing arterial run off Curves were obtained from a 46-year old normotensive woman a 54 year-old arteriosclerotic man without much diastolic hypertension a 56 year old severely hypertensive man treated with ganglionic blocking agents and hydralazine and a 37 year-old woman with untreated malignant hypertension The curves are a function of diastolic pressure (11)

seconds after occlusion from 30 to 122 mm Hg (average 65 8 normotension being 15 to 40 average 28 7 mm) a finding surprising on the surface but expected when due consideration is given to hypertensive hemodynamics The smooth muscle of all arteries and arterioles must therefore be in a state of chronic spasm otherwise hyperemia would occur in those which are not

Pathogenesis of Hemorrhagic and Exudative Retinitis The lesions found in the fundi oculi when the diastolic pressure is high are those of edema hemorrhage deposits of proteinaceous or lipid material and scarring Many ophthalmologists consider that hemorrhagic and exudative retinitis is due to localized ischemia of the retina secondary to excessive vasospasm From a hemodynamic anatomic and physiologic viewpoint this concept is hardly tenable since a) ischemia of a part does not usually cause edema without infarction b) ischemia does not lead to hemorrhage c) the retinal arteries and arterioles have rather *thin* muscular coats and d) the lesions appear when the diastolic pressure is high regress when it is lowered (sometimes to the point of producing retinal *ischemia*) and occur as a manifestation of a sudden worsening of the hypertension A more logical explanation is that of plethora or excessive hyperemia If the artery supplying an area of the retina were diseased so that it could not contract and healthy' vessels in the remainder of the body were made to constrict hyperemia through that diseased vessel *would result*. Excessive flow and pressure would be transmitted to the capillary bed supplied by that artery When venous outflow became insufficient to carry off the increased load *water* then plasma and finally red blood cells would be forced through the capillary wall This concept explains what we find edema cotton wool exudates and hemorrhages The hard

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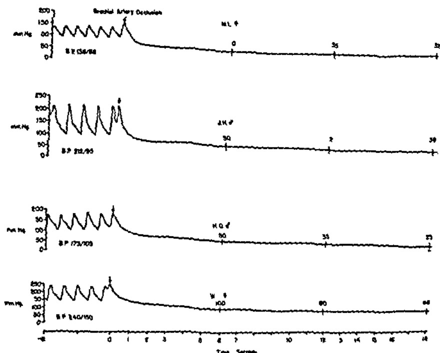


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Comment The secondary effects of hypertension are relatively unimportant to this discussion but they are of the utmost importance to the patient and to his therapy for it is by them that hypertension becomes a fatal disease A further and often fatal effect is discussed in Chapter VII

exudates may be the scars of old hemorrhages and the protein and lipid remnants of exudation of plasma

Since the arteries of the retina and brain are thin walled, weak structures compared to those in the remainder of the body, being in a media of higher external pressure, fairly generalized exudation and hemorrhage would be expected when systemic diastolic pressure rose to very high levels It is probable that focal hemorrhages in the kidney, and perhaps in the gut, are the result of a high diastolic pressure through an artery or arteriole which is so diseased that it cannot constrict as much as the rest of the vascular bed Therefore, many of the hemorrhagic and exudative lesions found in malignant hypertension are probably caused, not by excessive spasm proximal to the lesion but lack of enough spasm to compensate for that of the rest of the vascular bed

Pathogenesis of Necrotizing Arteriolar Lesions In man, we find necrotizing lesions of the arteries and arterioles usually of the kidney only in nitrogen retention (5) Two influences are necessary, azotemia and a high diastolic pressure Azotemia alone will not produce the lesions, for example, in uremia without much hypertension On the other hand, hypertension alone will not usually cause the lesions in dogs (6) or in man It is difficult to discover more than isolated cases in experimental animals without uremia (12) Both kidney (6) and a section of the small intestine can be protected from their occurrence by partial constric tion of the main artery even in severely uremic animals (13) Necrotizing fibrinous arterial degeneration however has been caused by the injection of organ, notably renal, extracts into nephrectomized dogs (14 15) Therefore both pressure and severe renal disease seem to be required for their occurrence

This form of reacting to stress may be common to some human beings in many environments and of many races although adequate studies have not been made The old idea that a part of the population is sympathotonic and part parasympathotonic or vagotonic may have some basis of fact. Different species of animals show different types of reaction to stress rats cats guinea pigs and rabbits not only exhibit opposite types but respond in aberrant ways to known pressor and depressor agents There are at least two kinds of dogs nervous overactive breeds which are hypertensive on the first and many subsequent examinations and more or less phlegmatic breeds or crossbreeds which exhibit normal blood pressures and bradycardia (21) There is little reason to believe that the human organism differs radically in its fundamental reactions from those of higher animals

Sympathotonic people are supposed to be subject to vasomotor phenomena tachycardia and cardiovascular diseases especially hypertension Parasympathotonic people are supposed to be subject to bradycardia a low blood pressure and gastrointestinal disorders especially peptic ulcer Another type of individual develops allergic reactions In any population all varieties and degrees of reactive ability can be expected depending probably on the amount of imbalance between sympathetic and parasympathetic nervous function and the amount of external stress to which individuals are exposed There may be several different constitutional types we have not observed true extrinsic asthma in a hypertensive person and such allergic states as hay fever and urticaria are much less common than in the general population duodenal ulcer is unusual in hypertension although it exists (4) rheumatoid arthritis and most malignant tumors are seldom encountered in a hypertensive population (22)

Chapter II

BASIC OR CONSTITUTIONAL FACTORS

THE BASIC factors in arterial hypertension are those broad and ill defined influences which cause a human being to become predisposed to the development of the disease By arbitrarily separating basic traits from factors operating after the disease has become established one can outline the areas of therapeutic approach and predict, with some success efficacy of various forms of therapy

Reaction to Stress by Vasospasm The basic defect in persons predisposed to hypertension appears to be a reaction to stress by vasospasm or through vasomotor phenomena Thus Levy, Stroud, White and Hillman (15 18) found in Army Officers that transient hypertension transient tachycardia and overweight each predisposed to the later development of hypertension the last factor being the least significant Long prior to these studies Hines found that when the stress of the first examination resulted in transient elevation of the systolic pressure to more than 140 mm Hg 63 per cent of the patients would develop hypertension 20 years later if more than 150 78 per cent would exhibit it (19) Critical predisposing diastolic levels were above 85 mm Furthermore Hines has shown that persons reacting by vasospasm to pain (cold pressor test) later usually develop hypertension (20) This manner of reacting therefore probably constitutes the underlying etiology, which lies in the constitution of the individual and is described but not understood

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The ability, or habit, of reacting to stress by vasospasm does not cause hypertension It merely predisposes those individuals so constituted to the slow development of the disease Other influences are probably operative which change reversible into irreversible vasospasm

HEREDITY AND ENVIRONMENT

Heredity The manner of reacting to stress by vasospasm is apparently an inherited characteristic Hines has shown clearly that persons who so react have parents who are either hypertensive or react likewise to painful stimuli (23) The gross hereditary nature of chronic hypertension is well established, although Pickering disagrees with this idea (24) what makes up this predisposition has been little understood

Thomas has clearly shown that cardiovascular disease is an inherited trait in the United States (25)

Personality Patients suffering from chronic arterial hypertension in all stages of severity supposedly exhibit certain defects of personality, the qualities of which go to make mature and well integrated individuals The deficiencies have been considered as a subnormal level of assertiveness and tendencies towards obsessive-compulsive traits (26, 4) Anxiety which may precede, or be a result of the illness, is also common (27) Whether these alterations are similar in the major psychosomatic disorders which result in irreversible organic changes (peptic ulcer, asthma) or are specific for one disorder, has not yet been clearly established Likewise the effects of abnormal renal metabolites (primary amines and other substances) upon cerebral metabolism, which might enhance relatively minor functional derangements, are not known (Chapter III)

Environment Without stimuli to cause a reaction the reaction would not occur The stresses, or stimuli, which

are a product of the environment and the attitude of the individual toward it can be considered as initiating factors in the reaction These influences vary widely from one person to another involving the many faceted aspects of existence and adjustment to a prevailing social order It is probable that the more complex society becomes the greater are the environmental stresses consequent to adjustment to that society and therefore the stimuli to somatic reaction increase

Pickering lately summed up his concepts on the relation of heredity constitution and environment in hypertension (28) To get these conclusions in perspective it may be said that in its mode of inheritance blood pressure resembles height but that the size of the genetic factor is greater in the case of height. However the regression coefficient certainly underestimates the size of the genetic factor since we have been unable to allow for the day to-day variability of blood pressure and we have had to allow for the effects of age by a device which is probably valid when it is applied to large numbers but not so accurate for individuals By contrast, height shows quite insignificant variations from day to day and for a considerable span of adult existence is uninfluenced by age The difference between the size of the genetic factor in blood pressure and height is probably less than regression coefficients suggest. Even so it would seem justifiable to conclude that environmental factors are more important than hereditary factors in the pathogenesis of hypertension

These considerations lead to one further idea which is so revolutionary that I merely lay it before you knowing that your minds must instinctively reject it namely that the current concept of essential hypertension as a specific disease entity is largely an artefact. I venture to suggest that a restatement of the facts would define essen

tial hypertensives as that group of the population with arterial pressures exceeding a certain value arbitrarily selected and in whom no specific cause can be detected to account for the high pressure It is suggested that the factors causing it are factors operating generally on the population Of these factors the contributions of age, sex and inheritance can be defined approximately The influence of environmental factors which would seem by exclusion to be of great importance, remains to be explored

We do not believe that this idea is so revolutionary, having entertained it for many years (29 31, 4) In Chapters V and VI will be discussed the factors operating generally on the population in Chapter III this curious, ill defined but well known vasomotor manner of reacting which varies from individual to individual These factors can now be examined separately

CLINICAL IMPLICATIONS

Since persons predisposed to hypertension emotionally react to environmental stresses through somatic pathways by vasospasm, *in the very earliest stages of the disorder* some reversal of the somatic response can be expected if reversal of one or more of the psychic components could be accomplished Many attempts to do so have been made Psychotherapy has been extensively employed in young individuals without organic disease it may teach the person either to avoid emotional stresses to sidetrack the reactions thereto along other pathways or to resolve them without somatic reaction by means of logic and insight In patients of older ages, with somewhat more advanced hypertension or with organic changes, however little in the way of therapy can be expected By analogy, while psychotherapy of peptic ulcer is useful to promote healing and to prevent further attacks it is useless in relieving

pyloric obstruction with scarring secondary to repeated attacks These defects of personality however are probably so deep-seated and fundamental to the growth of the individual that complete rebuilding becomes most difficult except in young people In our experience psychotherapy has failed to modify the course of severe hypertension sufficiently to allow us even to suspect some beneficial somatic effect and we have often watched patients deteriorate to eventual death in spite of the most vigorous forms available (an analyst reanalyzed by an analyst)

An environment considered unfavorable by the individual may be altered by moving to a new one temporary effects upon the course of moderate and mild stages have been observed The familiar fall in blood pressure when patients enter the hospital is an example How permanent this change can be is not known Minor adjustments in adverse environments especially those caused by other individuals with whom the patient is in close contact may for a time alter emotionally induced stresses (Figure 2)

Drugs especially sedatives have been employed for many years for the purpose of suppressing the emotional tension and lowering the threshold of reactions to stress As a general rule the more severe the *hypertension* the *less effective* are sedative drugs and other such influences upon the disease Contrariwise the milder the *hypertension* the more effective are measures aimed at the psyche and the emotional disturbance

The effects upon the course of hypertension of any one or combinations of the above approaches is directly proportional to the relative influence of these factors in the total picture Psychosomatic diseases may start as functional derangements mediated through autonomic nerves and end as organic conditions causing *death* Therefore while the beginning may lie in the psyche as exemplified by the

word, constitution, that factor becomes increasingly less important as somatic changes occur In reversible early and very mild stages the disease may be controlled, as its somatic ravages progress less and less can be expected from attacks upon these etiological factors Alterations of

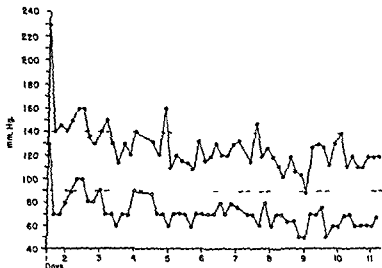


FIG 2 Typical rapid fall of blood pressure after hospitalization E E was a 39 year-old woman who was first discovered to have an elevated blood pressure six years previously at the time of pregnancy complicated by pyelonephritis During the intervening years her blood pressure was always elevated in a physician s office Six months and one month prior to admission she suffered two attacks of severe head aches numbness and weakness of one side of her body and loss of consciousness for one to three hours with gradual recovery during the following week Her blood pressure was said to have varied between 230/130 and 210/110 mm Hg for 2 years She complained of dyspnea on exertion and dizziness On examination there was one small retinal hemorrhage Blood pressure was 238/136 Her heart was enlarged to the left in x ray photographs Renal function was excellent No cause for the attacks was discovered on careful neurologic examination The first pressure shown (230/130 mm Hg) was measured by both the intern and assistant resident the night of admission subsequent ones were measured by nurses beginning the following morning (From Schroeder H A. and Perry H M Jr *Am Heart J*, *51* 776 1956)

disturbed emotional and nervous functions cannot be expected to dissolve scar tissue

Although Bays and Scrimshaw disagree (32) from all the evidence available we can be fairly certain that hyper tension not secondary to renal disease is a disorder fairly well confined to persons exposed to the influences of Western Civilization (4) For example it is unusual in parts of Africa (33) and China (8) very prevalent in Ameri can Negroes but rare in American Indians in the South west (34) In Uganda only 2 6 per cent of autopsied cases of heart failure were due to essential hypertension the same percentage to atheromatosis and none to coronary thrombosis renal hypertension however, accounted for 16 per cent (35) Surely one is led to conclude that en vironmental influences are of the greatest importance for in this country probably half the cases of heart failure are hypertensive in origin When viewed from this out look many discrepancies in the geographic incidence of hypertension fall into line

Comment There are three apparent facts upon which one can speculate

1 The predisposition to hypertension is inherited

2 There is an emotional overlay in the disease which may be either primary or secondary

3 The disease is confined more or less to civilized or partly civilized people without any particular ethnic cultural or social pattern suggesting that environmental factors such as food habits or contact with industrialized society plays an initiating role

Chapter III

NEUROGENIC EFFECTOR MECHANISMS

INTRODUCTION

While yet unproven it seems clear that the sympathetic nervous system is somehow relatively or absolutely overactive in prehypertensive and hypertensive states, especially when there is no demonstrable organic renal component The indirect evidence, suggesting rapid alterations in the nervous control of blood vessels is as follows

1 The blood pressure is labile and widely variable (Fig 3)

2 Traube Hering and respiratory variations in blood pressure are often marked (36 37) (Fig 4)

3 The vasospastic response to painful stimuli and to emotion is often exaggerated (20 38 41)

4 The pressor effects of central vasomotor stimuli such as inhalation of carbon dioxide and holding the breath is often increased (42-44)

5 Blocking sympathetic nerves by drugs or surgery abolishes many of these exaggerated vasospastic responses to pain and emotion (45, 46)

6 Drugs acting partly on the central nervous system lower the blood pressure more or less *(vide infra)*

7 Sustained hypertension can be produced in certain animals by interfering with sympathetic and cerebral nervous mechanisms (47 50)

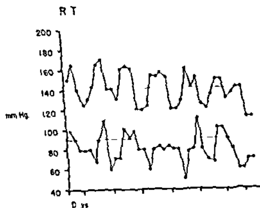


FIG 9 Daily and nightly fluctuations of supine blood pressure in an 18 year-old man in the "pre hypertensive" phase His kidneys and heart were normal by all tests Note that the rise in pressure occurs only during the day The divisions between each 24 hour period are at midnight

The evidence against neurogenic effector mechanisms operating in sustained human "neurogenic" hypertension is poor and usually explicable by an analysis of the cases employed for experimentation or by an understanding of the processes concerned in neurogenic vasoconstriction [#] At present no one doubts the existence of neurogenically induced vasospasm in man We must emphasize however that in chronic human arterial hypertension the relative parts played by neurogenic and other mechanisms vary considerably from patient to patient (Chapter IV) The contrary evidence follows

1 Little or no increase in urinary catechol amines is usually found (31) Norepinephrine however is liberated at nerve endings and metabolized or conjugated *in situ* before its products reach the blood stream Therefore overproduction must be great enough to saturate oxidative and conjugative enzymes in order to allow enough to spill

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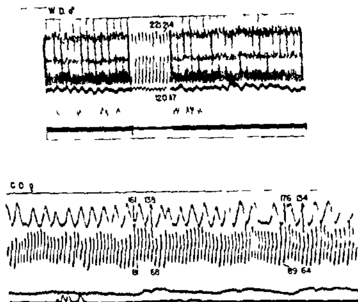
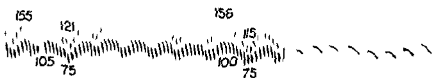
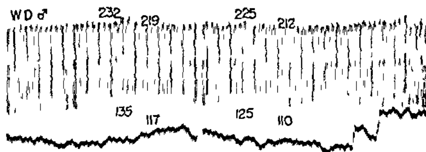


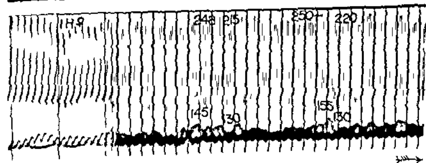
Fig 4 Spontaneous variations in blood pressure measured photokymographically by direct arterial puncture W G normal and normotensive variation 9 6 mm Hg F T normotensive convalescent from severe acute poliomyelitis with probable slight involvement of the hypothalamus or medulla giving rise to neurogenic vasomotor instability variation 41/20 mm Hg W D fairly severe nephrogenic hypertension variation 13/15 mm Hg I H severe neurogenic hypertension variation 35 25 mm Hg W D (repeat after several days rest) variation 7 3 mm Hg C O mild neurogenic hypertension variation 42/25 mm Hg By indirect measurement C O s systolic pressure when taken by a white coated physician varied from 180 to 240 mm and her diastolic from 150 to 100 mm nurses always obtained readings 20 to 30 mm lower The wide fairly regular tracings are those of respiration the smaller ones of a plethysmograph on the finger Camera speeds 1 25 5 and 25 mm per second


WG ♂
110
120
60
60

FT ♂


155
121
156
115
105
75
100
75


WD ♂
232
219
225
212
135
117
125
110


1H.9
248
215
250
220
145
130
155
130

Several influences arising in the brain can cause experimental hypertension such as noise (54) internal hydrocephalus (46) puncture of the third ventricle (55) tying off the arterial supply (49) all of which may interfere with blood flow or nervous function in a regulatory area Similarly in man increased intracranial pressure certain brain lesions of viral vascular or traumatic origin (fractures of the base of the skull encephalitis poliomyelitis) certain tumors and hypothalamic injury can lead to chronic neurogenic hypertension or its acute equivalent (56)

There are four possibilities to explain the cerebral role in human hypertension 1 The nervous temperament of the hypertensive person with the frequent finding of anxiety and frustration may initiate repetitive discharges through the sympathetic nervous system Subnormal assertiveness obsessive-compulsive traits and anxiety are said to be common to the hypertensive personality (26 27) This hypothesis has always been an attractive one but is unproven and most difficult to investigate with the tools at hand

2 The peripheral metabolic abnormalities associated with hypertension may cause stimulation of cerebral metabolism It is known that many primary amines cause central excitatory effects Amphetamine (Benzedrine) is a good example In fact Mann and Quastel (57) suggested that the central stimulant action of *dl* phenylisopropylamine (Amphetamine) is related to its inhibition of tyramine oxidation by amine oxidase in brain On this basis Fellows and Bernheim (58) examined a large number of structurally related salts in rats and found in many instances good correlations between central stimulation and cerebral amine oxidase inhibition Clinically the excitatory actions of epinephrine and a number of derivatives are well known we have observed profound and

over into blood and be excreted in urine Actually some patients do have moderately increased amounts in their urines (51b) about a third

2 No increase in circulating catechol amines can be detected (38)

3 Many patients do not show all of the typical exaggerated pressor responses nor the marked depression of blood pressure induced by drugs or surgery (52) The logical explanation is that another vasospastic process is largely operative in such individuals

4 Although neurogenic pathways effecting vasoconstriction are intimately connected it is necessary to examine each component of the sympathetic nervous system in the light of its role and of specific effector substances and chemotherapeutic agents Some of these are known some must be postulated The brain and its appendages exert a profound effect upon normal vasomotor tone and are probably involved in many forms of generalized vasospasm "As Starling said (53) No pathology will be adequate which does not take into account the sensitiveness *of the vasomotor centers to the changes in the circulation* Considerable information on pathogenetic factors can be learned from the actions of specific drugs more perhaps than by direct experiments

CEREBRAL MECHANISMS

The areas within the brain initiating or transmitting sympathetic discharges or regulating vasomotor tone are three the cortex the hypothalamus and the vasomotor center Just how these areas are involved in the hypertensive process is not known What is known however is that certain drugs modify their activities and partly affect the amount of peripheral vasospasm neurogenically induced

the hypothalmus or vasomotor center may initiate somatic sustained neurogenic pressor responses While such lesions have been found in some cases (64) and may account for the hypertension of some older persons there is no uniform correlation with all cases and this idea remains merely an attractive hypothesis

SPECIFIC DRUGS

Whatever the cause of the increased nervous excitability of hypertensive patients many agents have been used to counteract it and thus produce variable effects upon blood pressure depending upon a) the relative part played by the brain b) the effectiveness of the drug and c) the ability of the patient to tolerate side effects Sedatives have been used for many years in an attempt to allay tension and anxiety They will not be discussed since their employment is wide

Serotonin Antagonists Reserpine causes depletion of cerebral serotonin in the experimental animal (65 66) platelet serotonin is also reduced to a low level The net effect of this agent a chemical analogue of *yohimbine is* to produce on affect the equivalent of a prefrontal lobotomy (67) *Its locus* of action appears to be pre hypothalamic and subcortical the posterior hypothalamus wherein lie the sympathetic centers is partially blocked (68) The effect of the drug is cumulative requiring a week or two for oral doses to act maximally *although* rapidly excreted the drug itself leaves serotonin *acceptors* in the brain blocked for long periods Aside from its tranquilizing action the net somatic effect is that of relative parasympathetic overactivity *(Table I)* Diarrhea and gastric hyperacidity can result peptic ulcer has appeared *de novo* or become activated in one of *our cases* chronic ulcerative colitis has developed (69) Various cerebral symptoms are

uncontrollable anxiety induced by intravenous isoamyl amine in laboratory workers, for example Therefore, some circulating primary amines may induce cerebral stimulation Fast diffuse dysrhythmias in the electroencephalograms are common in human neurogenic hypertension (4) this picture can be produced by certain amines (59) Thus, a vicious circle could be established, from periphery to brain to periphery, the initiating organ not being known

Serotonin a derivative of tryptophane, has received the greatest interest in this regard, since its isolation from platelets This primary amine occurs in brain and may have a definite function in nervous tissue (60), as may other similar substances It is interesting and perhaps more than coincidental, that malignant carcinoid of the appendix, a serotonin producing tumor, apparently causes a peculiar flushing phenomenon which is similar to the diencephalic blush which we associate with neurogenic hypertension (61, 62) Injection of serotonin in man causes a variety of subjective symptoms not apparently associated with anxiety but similar in some respects to those seen following other substituted primary amines (63) The role of several tertiary and quaternary nitrogenous compounds on nerve conduction and synaptic transmission is barely beginning to be appreciated

3 There is either excessive production of stimulating substances *in situ* or generalized inhibition of those enzymes concerned with metabolizing such substances For example if every sympathetic nerve ending contained a molecule inhibitory to amine oxidase or to the enzyme conjugating norepinephrine the normal tone of sympathetic nerves would be enhanced There are no proofs of this theory

4 Local vascular lesions of an arteriosclerotic nature in

TABLE I—(*continued*)

	Reserpine (123 subjects)	*Chlorpromazine (137 subjects)*
Epistaxis	2 4	0
Blurred Vision	0	1 5
Dry Mouth	0	1 5
Heart Burn	0	1 5
Edema	7 3	5 1
Pruritus	1 6	1 5
Dermatitis	0	9 5
Jaundice	0	5 1
Hepatomegaly	0	2 2

also induced among them vivid dreams and nightmares One of the most interesting of its actions is to cause, in a sizeable percentage of people nervousness insomnia agitated depressions and suicidal tendencies (70) used to treat these symptoms in psychotic individuals it can backfire and produce them Truly this most interesting drug has begun to open up a wide field in our understanding of mental illness

Chlorpromazine is also an antagonist to serotonin (73) Its locus of action has been presumed to lie subtentorially probably in the medulla tranquilizing effects are seen as well and hypothalamic action has been postulated (74) While it can cause some depression of the sympathetic nervous system it can also produce symptoms of stimulation in some individuals (Table I) with hypertension and tachycardia Likewise its antiemetic action may be reversed in other subjects

Other antimetabolites to serotonin are not used in hypertension yohimbine because of its nephrotoxicity and d lysergic acid diethylamide which produces schizophrenic like states (63 66) The most interesting are the nitroindoles which are true competitive antagonists blocking

TABLE I

RESFRPINE AND CHI ORPROMAZINE
INCIDENCE OF SIDE REACTIONS AND TOXIC EFFECTS IN NORMOTENSIVE PATIENTS

	Reserpine (*123 subjects*)	*Chlorproma ine* (*137 subjects*)
Sympathetic N S Inhibition		
Hypotension	31 6	14 6
Bradycardia	17 0	3 7
Diaphoresis	1 6	0
Chilliness	3 3	0
Nausea	5 7	7 3
Vomiting	2 4	5 1
Diarrhea	7 3	0 7
Exacerbation of Peptic Ulcer	0*	0 7
Sympathetic N S Stimulation		
Hypertension	0	6 6
Tachycardia	0	8 8
Hyperthermia	0	3 7
Cerebral Symptoms		
Excessive Flushing	9 0	0
Dizziness	5 7	5 1
Fatigue neakness	9 7	2 2
Syncope	1 6	0 7
Excessive Drowsiness	17 8	12 4
Tremulousness	15 4	0 7
Myalgia	3 3	0
Ataxia	5 7	0
Parkinsonism	1 6	0 7
Vivid Dreams	2 4†	2 2
Agitated Depressive Psychosis	±5‡	
Other		
Nasal Stuffiness	27 6	1 5

* We have seen 4 cases

† Higher in our experience

‡ Author s series

(*From Zeller* W W Graffagnino P N Cullen C F and Reitman H J U e of chlorpromazine and reserpine in the treatment of emotional disorders *J A M A* *160* 179 1956)

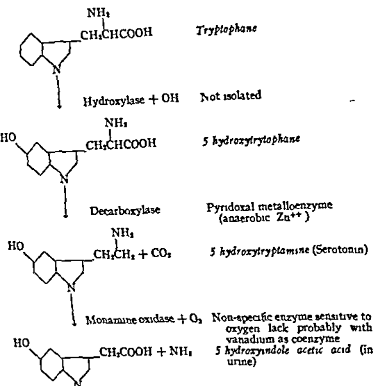


Fig 5 Metabolism of serotonin, modified from Sjoerdsma *et al* (61)

phane and its decarboxylation (Fig 5) The hydroxylase has not been discovered but the renal decarboxylase (71) contains a pyridoxal metal complex as a coenzyme (72) like many other amino acid decarboxylases. Serotonin is metabolized by monamine oxidase (Chapter IV) an enzyme requiring oxygen and sensitive to oxygen tension Therefore renal ischemia could allow the formation of serotonin by anaerobic decarboxylation but prevent deamination *in situ* due to oxygen lack Serotonin would then escape into the blood and be deaminated either in the lungs or on arterial smooth muscle While it is doubt

the action of serotonin on smooth muscle, they have not been employed more than sporadically with little effects

A newer sedative, 2 methyl 2 n propyl 1,3 propanediol dicarbamate (meprobamate) apparently selectively blocks interneurons primarily on the thalamus and caudate nucleus (75) There is little or no effect upon the autonomic nervous system This drug should therefore prove a tool for controlling anxiety in mildly hypertensive patients and thus estimating the role which nervous tension *per se* plays in minor elevations of blood pressure

Comment None of these agents are more than mild antihypertensive drugs One or another may control moderate or intermittent elevations of blood pressure, especially when associated with emotional tension, but they are relatively valueless, except as adjuncts, in more severe cases Obviously the sustaining mechanism for severe hypertension lies elsewhere than in the brain, although *initiating* mechanisms may be there, in cerebral edema, however, a large neurogenic influence may be exerted The most potent and specifically acting drug can do no more than inhibit the relatively minor role which the brain contributes to the process of generalized vasospasm Even after destruction of much of the brain by atherosclerotic disease, to the point of causing a vegetative existence, established hypertension may not disappear

Serotonin is one of the newest agents discovered to be involved in cerebral interneurone transmission Of great interest is the fact that this primary amine apparently has a specific affinity for cortical pathways to the posterior and lateral hypothalamus (Too much could stimulate and cause emotional tension a normal amount interest, initiative and drive, while too little could result in mental depression) Serotonin is found in quite primitive marine organisms, it is formed by the hydroxylation of trypto-

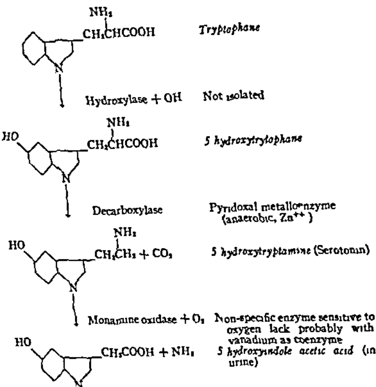


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ful that serotonin is concerned directly in hypertension, injections into hypertensive, but not normotensive animals and man are moderately pressor, and this interesting substance in slightly increased amounts could represent an abnormal metabolite causing some symptoms and a small portion of the vasospasm

CAROTID SINUS MECHANISMS

There is no good evidence that the carotid sinus mechanism is underactive in the usual case of hypertension Chronic sustained hypertension can be produced in dogs however, by ablation of the carotid sinus and aortic depressor nerves (76) while this state is true neurogenic hypertension, after several years the renal lesions of arteriolar nephrosclerosis develop (7) In our experience, this form of hypertension is not dependant solely upon increased cardiac output as there is intense peripheral vasoconstriction under anaesthesia (10) A clinical counterpart may be suspected in patients with atherosclerotic narrowing of the mouths of the innominate and left common carotid arteries That an appreciable degree of narrowing is uncommon may be suspected from the clinical observations *that symptoms of severe cerebral ischemia do not* usually follow reduction of elevated blood pressure to normal that some degree of obstruction may occasionally be found is suggested by the vague discomfort accompanying normotension seen in many atherosclerotic hypertensive individuals (While hardening of the carotid sinus wall and local atherosclerotic narrowing have been suggested (77) these lesions are unproven as causes of chronic hypertension)

Specific Drugs There is a group of drugs from plants of the veratrum family which do affect the carotid sinus *sensitizing the pressor rereptors and thus increasing the* activity of the depressor mechanism (78) Protoveratrine

A and B are the most purified alkaloids usually found in a mixture and most difficult to separate They cause depression of blood pressure and bradycardia nausea and vomiting may result from vagal stimulation (79) The pathway is through the glossopharyngeal nerve to the vasomotor center (78) When doses are adjusted properly intermittent normotension can result from the careful use of protoveratrine and its impure derivatives (80) Apparently tolerance is quick to appear and disappear so that sustained normotension will not result unless adjuncts operating on other mechanisms are used Whether or not this drug is a true antihypertensive agent affecting the basic process is unclear although the hemodynamic response is quite favorable (Table II)

Baroceptor Changes McCubbin Green and Page (81) have recently shown that the carotid sinus and aortic depressor mechanisms are set at a higher level of pressure in renal hypertensive dogs than in normotensive dogs They propose the ingenious theory that this higher setting maintains the hypertension even when the initiating mechanism (renal ischemia pheochromocytoma toxemia of pregnancy) is removed Thus renal hypertension slowly becomes neurogenic buffer nerve hypertension as Ogden has suspected in rats (82) If this were so in man one would expect that late chronic hypertension would respond to the use of drugs or surgery acting on nerves better than would early hypertension Clinically the opposite holds true therefore this attractive hypothesis necessarily can be discarded as applying to most human cases

SYMPATHETIC NERVOUS MECHANISMS THROUGH GANGLIA

All autonomic nerves after emergence from the spinal cord pass through ganglia In general sympathetic nerves

TABLE II

CARDIOVASCULAR EFFECTS OF PROTOVERATRINE AND GANGLIONIC BLOCKING AGENTS IN MAN

	Normal		*Hypertensive*	
	Epineph rine	*Norepineph rine*	*Proto-veratrine*	*Gan glionic Blockade*
Cardiac				
Heart Rate	+	–	–	–
Stroke Volume	+	+	+	–
Cardiac Output	+	0	0	–
Coronary Blood Flow	+	+	?	?
Blood Pressure				
Systolic Arterial	+	+	–	–
Mean Arterial	+	+	–	–
Diastolic Arterial	–	+	–	–
Mean Pulmonary	+	+	?	–
Peripheral Circulation				
Total Peripheral Resistance	–	+	–	–
Cerebral Blood Flow	+	0–	0	0
Muscle Blood Flow	+	0–	+	+
Cutaneous Blood Flow	–	+	?	+
Renal Blood Flow	–	–	0	–*
Splanchnic Blood Flow	+	0	?	–

* *Transient*

thetic nerves end in ganglia at more peripheral (organ) areas The adrenal medulla receives preganglionic fibres which cause discharges of epinephrine a *sympathetic effec tor* substance, into the circulation therefore it may be considered a ganglion in the broadest sense of the term

The chemical mediator of ganglionic transmission is a quaternary ammonium compound acetyl choline Whether or not other compounds containing tetracovalent nitrogen

or choline esters can act as transmitters is not known Acetyl choline (or its derivatives) apparently is essential for synaptic transmission in all ganglia both sympathetic and parasympathetic. Nicotine in small doses is a stimulant.

The ganglion itself governs the integrity of the post ganglionic fibres much as the spinal nuclei control the integrity of their neurons. Removal of a ganglion is probably followed by degeneration of the nerve after a few days sensitivity of the nerve ending to circulating vasoconstrictor substances develops Therefore in order to perform an adequate sympathectomy preganglionic fibres must be cut

Specific Drugs Chemical ganglionic blocking agents usually contain quaternary ammonium stabilized tetra covalent nitrogen competing with acetyl choline or other more labile nitrogenous substances Numbers of such compounds exist The simplest one of the group is tetraethyl ammonium ion known for many years as a vasodilating drug of short action Longer action is achieved by lengthening the carbon chain and doubling the nitrogen group (pentamethonium pendiomide hexamethonium) or by adding cumbersome ring structures (pentolinium chlorisondamine) All act in a similar manner differing only in duration of action and degree of gastrointestinal absorption A new blocking agent mecamylamine (Inversine) differs considerably in structure being a complex spatial molecule with trivalent nitrogen as a secondary amine It has the advantage of virtually complete absorption from the gastrointestinal tract (83) Comparative doses are shown in Table III (Fig 6)

Since acetyl choline also mediates nerve transmission to striated muscle it may appear strange that curariform

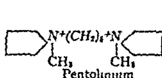
paralysis does not result from ganglionic blocking agents Some anatomical or chemical differences between ganglia and motor end plates undoubtedly exist, for hexame



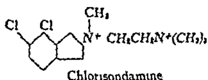
Tetraethyl Ammonium (TEA)



Pentamethonium (C_5)



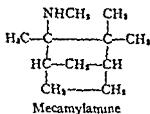
Pentolinium



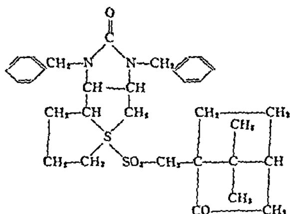
Chlorisondamine



Hexamethonium (C_6)



Mecamylamine



Trimethaphan Camphor Sulfonate

FIG 6 Structural formulae of ions of some ganglionic blocking agents The salts are not shown The last known as Arfonad is only used intravenously Note the quaternary or tetracovalent nitrogen groups which are the active ones and the camphane structure of those with trivalent nitrogen

TABLE III

COMPARATIVE DOSES OF GANGLIONIC BLOCKING AGENTS (SEVERE ADULT HYPERTENSION)

Ion	*Usual Effective Oral Dose*		*Maximum Tolerated Oral Dose*		*Duration of Action of Oral Drug*	*Usual Effective Parenteral Dose*‡
	Single Dose mg	*Daily Dose mg*	*Single Dose mg*	*Daily Dose mg*	*hrs*	*Single Dose mg*
Tetraethyl Ammonium	†	†	†	†	½–1	500
Pentamethonium	500	2500	?	?	3	25
Hexamethonium	500	2500	1000	6000	4	25
Pentolinium (Ansolysen)	100	500	800	4000	4–5	25
Chlorisondamine (Ecolid)	50	250	200	1000	4–6	15
Mecamylamine (Inversine)	10	50	25	150	4–8	10

* When combined with hydralazine

† Sublingual doses effective oral not

‡ *Initial* dose much smaller Wide variation as tolerance develops

thonium ion in large doses can exhibit curare like actions Lengthening of the carbon chain to 10 atoms results in a curariform drug, decamethonium curare itself contains tetracovalent nitrogen, and succinyl choline has a similar action (84) The longer the chain from 5 to 10 carbon atoms, the greater is the paralytic effect

The cardiovascular effects of ganglionic blockade are summarized in Table II Thus, ganglionic blockade while lowering blood pressure, does not act primarily upon all of the functions disturbed in hypertension Furthermore, sympathetic nervous inhibition at the ganglionic level is also associated with parasympathetic nervous inhibition Renal and splanchnic blood flow are altered in the wrong direction Continuation of these disturbances could cause serious consequences, were it not for some unknown re adjustments which take place within the organism counter acting the changes While the effects of these agents in opposing the hypertensive process are real and offer evi dence for the role of the sympathetic nervous system in pathogenesis, they are not all to be desired Presumably they differ from the effects of surgical sympathectomy The subject of the specificity of these drugs on the basic proc esses concerned in vasospasm is open and arguments pro and con the question of whether or not the observed effects are truly antihypertensive have validity on both sides

SYMPATHETIC NERVE ENDINGS

The chemical effector substance of the sympathetic nerves is norepinephrine On stimulation of a nerve, this primary amine is released at the junction of nerve and organ or smooth muscle fibre Infusion of norepinephrine intravenously mimics the cardiovascular profile seen in sustained arterial hypertension (Table II) and a similar

"picture is caused by norepinephrine secreting pheochromocytomata Therefore the neurogenic component of hypertension can be considered to be mediated by this substance '

Norepinephrine is derived either from dihydroxyphenyl serine by decarboxylation or from tyramine by hydroxylation of the benzene ring and the β-carbon The first appears the most facile method for the nerve ending to make this substance rapidly It is inactivated either through conjugation through oxidation of the amine nitrogen by monamine oxidase or by rearrangement of its molecule to form an indole nucleus through oxidation by poly phenol oxidase a copper enzyme

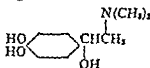
The ideal agent for counteracting norepinephrine has not been found There are a number of sympatholytic drugs which inhibit its action on nerve endings and which are effective for short or longer periods in experimental animals We list them only as directions for research These may be grouped roughly as derivatives of benzylamine of phenethylamine of ergot of benzodioxane and of imidazole (Table IV see page 48) All contain tertiary substituted nitrogen

Derivatives of Benzylamine Dibenamine a complex structure remotely related to norepinephrine forms tight bonds at sympathetic nerve endings preventing the action of this constrictor substance probably by competitive *in hibition* The action is prolonged for many hours It is moderately effective by mouth much more so intravenously There are many side effects in man especially on the brain Dibenamine and its *relatives* are *the most* effective sympatholytic agents known at present but their value in hypertension remains to be proven

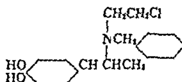
Derivatives of Phenethylamine *We had* an opportunity of testing a group of primary amines in rats for sympath

olytic qualities, some of which were given intravenously in man

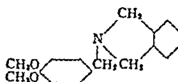
Those blocking norepinephrine in the rat had the following formulae



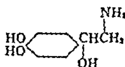
SKF 1298 A



SKF 669 C

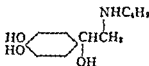


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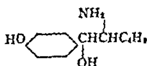


Norepinephrine

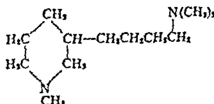
The nitrogen in these compounds was completely substituted all being tertiary amines There was, however no consistency in the results for the following gave no blocking action



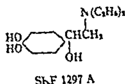
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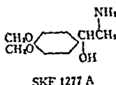
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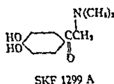
51 143



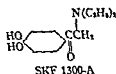
SKF 1297 A



SKF 1277 A



SKF 1299 A



SKF 1300-A

Renal hypertensive dogs responded by a lower diastolic pressure only when SKF 1298-A was given * SKF 690 A was a powerful epinephrine like substance in man causing vasodilatation and an increased cardiac output with fall in diastolic pressure SKF 1298-A caused symptoms suggestive of cholinergic stimulation two others were without effect

The experiments of Furchgott are of interest Using the spirally cut rabbit s aorta as a source of smooth muscle he was able to show on this simple system how certain agents such as dibenamine block all constrictor amines others block some and others block only a few (85) Therefore it is likely that very specific agents can be found which will pick out one primary amine and not others for inhibition

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* None of these substances specifically depressed elevated blood pressure in the anaesthetized renal hypertensive rat without affecting normotensive rats.

TABLE IV

Actions of Adrenergic Blocking Agents in Man*

Function	*Diben.yline*	*Dihydrogenated Ergot Alkaloids*	*Phentolamine and Tolazoline*	*Ben odioxanes*
Principal Action on Epi or Norepinephrine	Both	Epinephrine	Both	Epinephrine
Cardiovascular				
Cardiac Output	Unchanged	Unchanged or Decreased	Unchanged	Decreased?
Blood Flow—Femoral	Increased	Increased	Increased	Unchanged
—Mesenteric	Unchanged	Decreased	Decreased transiently	?
—Renal	Unchanged	Decreased	Decreased	?
—Cerebral	Unchanged	Unchanged		
Coronary arteries	Dilated ?	Constricted	Dilated	Constricted
Peripheral Resistance	Decreased	Decreased	Decreased ?	Variable
Principal Adverse Effects				
Central Nervous Stimulant	+	+	0	+
Tachycardia	+	+	+	+
Tissue injury local	+	0	0	0
Nausea and Vomiting	+	+	0	0
Duration of Action	Days	Hours	Minutes	Minutes

* After Goodman and Gilman (84)

Derivatives of Ergot The dihydrogenated ergot alkaloids have the ability of blocking adrenergic impulses but their action is greater on epinephrine than on norepinephrine Toxic effects limit the tolerable dose so that useful blockade is rarely produced They must be given sublingually or parenterally their effects in hypertension vary but are usually inconsistent.

Derivatives of Imidazole Tolazoline (Priscoline) and Phentolamine (Regitine) are two short acting moderately effective adrenergic blocking agents containing both benzene and imidazole rings Phentolamine while opposing the actions of both epinephrine and norepinephrine is useful for the most part only as a test substance for circulating catechol amines It usually causes a transient fall of blood pressure in hypertensive patients suggesting that some sympathetic tone is present in azotemia the effect may be prolonged and profound Cardiac stimulation is the rule Tolazoline is readily absorbed and excreted unchanged in the urine phentolamine is apparently metabolized up to 90 per cent of the dose The short durations of action limit their use

The Benzodioxanes Piperoxan (Benodaine) and prosympal first synthesized by Fourneau act transiently usually by vasoconstriction They do oppose however the action of epinephrine probably by *competitive inhibition* norepinephrine is blocked only by toxic doses Side effects are many especially smooth muscle stimulation and limit their use except in epinephrine producing pheochromocytomas

Comment All of the known adrenergic blocking agents fail to block cardiac accelerator mechanisms tachycardia and increase in cardiac output of reflex origin limit their clinical uses In all cases blockade of injected or circulating

vasoconstrictor amines is greater than is the blockade of nerve impulses, dibenamine has less differential activity in this respect The ideal agent for chemical sympathectomy is one which prolongedly blocks norepinephrine at all vascular nervous endings including especially those in the heart *This agent has not been found*, if it can be it should prove the best agent for controlling the neurogenic factor in human hypertension

Other Effector Substances While the role of other sympathomimetic amines in hypertension is not established they are probably present in excessive amounts and may contribute to symptoms if not to vasospasm Decarboxylation of amino acids by kidney is an anaerobic process (86, 87) while deamination is an aerobic one (88) the enzymes monamine oxidase and possibly diamine oxidase being sensitive to oxygen lack (89) Under these conditions any amino acid decarboxylated by the kidney could form amines by partial interrupted metabolism, altering the locus of deamination from kidney to peripheral smooth muscle or liver The level of primary amines in hypertensive blood is usually high (4 90, 91) This error of metabolism will be discussed at length in Chapter IV

CLINICAL IMPLICATIONS

In the absence of a good specific adrenergic blocking agent which also blocks the cardiac sympathetics we are forced to use combinations of drugs depend upon ganglionic blockade or affect the carotid sinus mechanism Combinations of adrenergic blocking agents such as dibenamine derivatives and protoveratrine have been advocated the former to block nerve endings and the latter to slow the heart In fact one such preparation also contains reserpine, which tends to cause bradycardia Such

pousse café combinations are to be avoided all drugs give reactions and side effects and it would be difficult to assess the vomiting induced by dibenamine and that by protoveratrine in such a mixture

The toxic or side reactions of reserpine and chlor promazine have been given in Table I The most serious late toxic reactions of reserpine are those of agitated depressive psychosis which are often accompanied by suicidal tendencies and may lead therefore to death Of chlorpromazine there are hepatic disease and granulocytopenia some 17 deaths have resulted (92) Chronic administration of any drug given to control not cure a chronic disease may back fire Furthermore in severe hypertension the use of mild drugs is potentially dangerous giving the physician a sense of security while the disease continues relentlessly on its ravaging course

There are no known late toxic reactions to protovera trine Immediate side effects are those attributable to vagal stimulation i e nausea and vomiting The rapid development of partial tolerance in a few hours with restoration of sensitivity after a few hours rest is unexplained

Ganglionic blocking agents show many side effects most of them the result of parasympatholysis or sympatholysis (Table V) Only two serious ones of this nature have been encountered The first occurs when partial often asymptomatic obstruction to a hollow organ has been present Complete obstruction may result The second is concerned with the mode of excretion Absorbed blocking agents are excreted in the urine If severe renal disease is present ganglionic blockade may cause hypotension and anuria as the drug is then retained hypotension and anuria persist

To set one pharmacologic thief to catch another pharma cologic offender is undesirable in modern chemotherapy

but sometimes it becomes necessary The activity of cholinergic drugs is enhanced when ganglia are blocked, thus, urecholine and prostigmine provide useful tools in abating unwanted parasympatholysis (98) Likewise norepinephrine infusions combat the hypotension quite effectively

TABLE V

SIDE EFFECTS OF GANGLIONIC BLOCKADE

Carotid Sinus Reflex	Decreased
Cardioaccelerator nerves	Blocked
Cardiovascular reflexes (cold pres or etc)	Blocked
Venous pressure	*Decreased*
Eye—Pupil	Fixed in mid position
—Accommodation	Fixed at normal resting point
Ptosis	Slight or absent
Ear—Eustachian Tube	Paralyzed?
Salivary secretion	Decrea ed
Gastric juice acidity and volume	Decreased
Gastrointestinal motility	Decreased
Gastric tone	Decreased
Colonic tone	Decreased
Defecatory Reflex	Decreased
Urinary Bladder tone	Decreased
Sweating	Decreased
Sexual potency (male)	Inhibited
Response to injected norepinephrine	Increased
Response to injected cholinergic drugs	Increased

Mecamylamine intoxication occurs in *azotemic* individuals and in others with poor renal function It is characterized by gross, generalized coarse muscle tremor, increased with activity, disappearing with sleep, by nervous tension and sometimes by visual hallucinations The state resembles delirium tremens The flapping tremor, which involves all voluntary muscles in advanced stages, is not associated with cog wheel rigidity and only moderate hy

perreflexia is found The tremor usually remains for many days after discontinuation of the offending drug even as long as two weeks Severe hyperpyrexia without infection leading to death was observed once Dilantin may partly ameliorate the condition It is probable that this secondary amine of a camphor nature affects the central nervous system camphor itself is convulsant and high doses of mecamylamine cause gross tremors in dogs We have observed fine tremors occasionally when hexamethonium ion was used Meprobamate can cause leucopenia

Ganglionic blockade disease occurs in poorly treated malignant hypertension (93 94 5) it is characterized by excessive tachypnea worsened in the sitting or standing position diffuse or patchy roentgenologic changes in the lungs with few physical signs and interstitial pulmonary fibrosis at autopsy Almost all cases have exhibited azotemia (5) the microscopic findings are indistinguishable from uremic pneumonitis One patient recovered after the use of cortisone the remainder died

All of the antihypertensive agents with powerful actions can induce cardiovascular accidents due to the nature of the arterial disease (atherosclerosis) often encountered and too sudden alteration of hemodynamics Arterial thrombosis is the most serious although it is rare Such reactions are not true side effects of the drugs themselves but are inherent dangers in their overenthusiastic and careless use

Summary Because drugs acting specifically on the autonomic nervous system may affect the elevated blood pressure in human hypertension we may assume that there can be a profound neurogenic component in some cases This component probably is mediated via sympathetic nerves Although the ideal counteracting agent is not avail

able, certain tools can be used with varying results on the course of the primary disorder All have side effects and most, late toxic reactions, which usually do not preclude their use provided careful attention is paid to the patient and his personal reactions

Chapter IV

NEPHROGENIC EFFECTOR MECHANISMS

EVIDENCE FOR EXISTENCE OF OTHER EFFECTOR MECHANISMS

To this point we have inferred that mechanisms other than neurogenic account for much of the generalized vasospasm seen in severe hypertensive states Although their natures are imperfectly understood there is sufficient experimental and clinical data to warrant careful examination of several hypotheses which fit or do not fit the facts

Most of the evidence for the existence of effector mechanisms other than neurogenic comes from experimental hypertension and from the wide variations in the acute or prolonged effects of drugs acting on sympathetic nerves To take up the pharmacologic evidence the following clinical observations are pertinent

1 Early and mild hypertension responds well to simple measures and milder acting sympatholytic drugs severe hypertension little or not at all

2 Extensive surgical sympathectomy either lumbodorsal or subtotal still leaves a sizeable proportion of patients as hypertensive as before relieves a fair number completely with the remainder improved to variable degrees

3 Full therapeutic doses of ganglionic blocking agents or protoveratrine cause intermittent or sustained normotension in a few cases a modified response in many and no appreciable effects (other than postural ones) in the more severe forms of hypertension

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4 Injection of tetra ethyl ammonium ion causes transient falls of blood pressure to a floor, this level being low in milder cases and rising as the disease progresses into severe stages Only in cerebral edema does the floor fall (95)

5 Injections of hexamethonium ion in full doses cause variable responses the estimate of the neurogenic component affected by the drug ranging from 100 to 15 per cent of the total elevation of blood pressure above normal The less the fall after hexamethonium ion, the higher is the final blood pressure There is a reciprocal relationship between neurogenic and humoral factors in maintaining the blood pressure high (53) (see Table XIX, p 110)

THE NATURE OF THE OTHER MECHANISMS

Two processes can be hypothecated to explain these findings

1 The arteries and arterioles become so sclerotic that a mechanical increase in peripheral resistance accounts for the sustained hypertension in the absence of neurogenic mechanisms This explanation is incompatible with the anatomic and pharmacologic facts While hypertension causes vascular lesions, they vary in intensity and degree throughout the body Only late hypertension is associated with these lesions Reduction in blood pressure of a degree sufficient to cause local ischemia in areas of severe vascular disease usually can be accomplished without such ischemic manifestations

2 The arteries and arterioles are in a state of spasm which is not mediated through nervous mechanisms This is the only tenable hypothesis If so several causes of the spasm must be examined

a) Some organ is forming and discharging into the circulation abnormal substances, which either are strong

vasoconstrictors themselves or which inhibit the destruction of normally circulating pressor substances

b) Some organ is not destroying or excreting pressor substances normally present, so that they accumulate to form a new homeostatic level

c) Some organ is sensitizing the blood vessels to normally circulating pressor substances

d) For some reason the arterial and arteriolar walls become edematous thereby increasing peripheral resistance.

Probably all of these mechanisms can operate under different clinical circumstances

The vast experimental and large clinical experience with hypertension induced by renal ischemia focuses attention upon the kidney as a mediating mechanism for that component of elevated arterial pressure which is not neurogenic in origin The posterior pituitary however forms a pressor substance and the adrenal cortex can sensitize blood vessels to vasoconstriction therefore endocrine mechanisms must also be considered (Chapter V) In this section we are concerned however with nephrogenic mechanisms

First the effects of sympathetic nervous discharges upon the renal circulation must be examined Both emotional tension and catechol amines cause renal vasoconstriction abolished in the case of the former by sympathectomy Curiously enough norepinephrine constricts in so far as is known only the renal circulation to a greater extent than other vascular beds The hemodynamic profile is similar to that seen in hypertension with efferent arteriolar constriction being dominant Epinephrine produces the same renal profile Therefore increased neurogenic sympathetic tone can cause relative renal ischemia but ischemia of no other known organ

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a) Some organ is forming and discharging into the circulation abnormal substances which either are strong

4 No renin or angiotonin can be found after several weeks of hypertension although the enzyme is present at first VEM in blood however increases with time to a plateau (103)

5 The oxygen consumption of the kidney may be reduced (104)

In kidneys removed from hypertensive rats and dogs the following enzymatic alterations have been demonstrated

1 Amino acid oxidation is reduced (104 105) suggesting a general inhibition of oxidative enzymes

2 Transamination is reduced in the presence of adequate pyridoxal phosphate (104) suggesting a depletion from renal tissue of apotransaminase

3 Deamination of amines is reduced (104) suggesting depletion of monamine oxidase

4 Succinic dehydrogenase and possibly cytochrome oxidase are reduced (105) All of these enzymatic alterations can be explained by loss of renal tissue consequent to prolonged ischemia

In man the following changes have been measured

1 Renal oxygen consumption is usually reduced (106 107) reflecting the ischemia

2 The urine is usually acid (108) reflecting perhaps the acidity of the cortex in ischemia

3 There is a tendency for renal loss of sodium and some chloride (109 110 4) caused in the case of sodium possibly by the acidity producing loss of base

4 The ratio of ammonia to titrable acid is lower than normal influencing possibly the sodium losing tendency of hypertensive kidneys (111)

5 Primary amines in arterial blood are usually elevated (90 91 112) a result perhaps of insufficient deamination from oxygen lack

Removal of one ischemic kidney before hypertension

Second, what metabolic abnormalities are present in renal ischemia? This subject is little understood and alterations little measured We know of some functional changes in experimental animals Certain urinary abnormalities occur, reflecting what appear to be minor renal derangements The cause is reduced blood flow, but whether it is mediated through oxygen lack or through some other mechanism concerned with flow remains to be discovered In anaesthetized experimental animals the following occur after acute mechanical constriction of a renal artery

1 Cortical oxygen tension falls only to rise again without changing the constriction (96) suggesting intrarenal vasodilatation

2 The same changes in blood flow take place At this point the renal vascular bed becomes sensitive to injected epinephrine (97)

3 The cortex becomes acid (96)

4 Renin the renal proteolytic enzyme is released into renal venous blood where it reacts with a globulin to form hypertensin or angiotonin a constrictor peptide (99) Likewise, a vaso excitor material (VEM) appears in blood (100) Angiotonin produced by the ischemic kidney, can constrict the vessels of the kidney making it more ischemic (101)

5 After several hours blood pressure may rise in the experimental animal (97), probably due to the release of renin and/or pressor amines

In the dog made hypertensive by partial constriction of a renal artery the following changes have been seen

1 Renal blood flow may be unchanged or decreased (102), but all of the increased resistance is not provided by the mechanical clamp there is a component of intrarenal vasoconstriction as well be it neurogenic or humoral (9)

2 Oxygen tension is lower than normal (96)

3 The cortex is acid (96)

This is more than an academic point If hypertension were the result of chronic inhibition of a renal enzyme removal of the enzyme or removal of the kidneys would accomplish the same result. The next question is whether a precursor is altered by ischemic kidney into a pressor

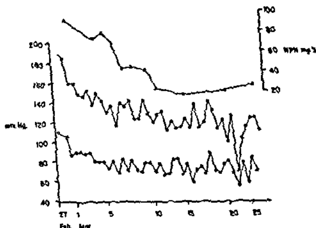


Fig 7 Azotemic hypertension with reversal when azotemia regressed Patient was a 45 year-old woman with abdominal lymphosarcoma which had involved both ureters *causing* bilateral hydronephrosis It was impossible to pass a *urethral* catheter through the left Radiotherapy was instituted, *resulting in a shrinkage of the* tumor a return of renal function toward normal *and a fall of* blood pressure occasionally to hypotensive levels

substance or whether normal kidney inactivates a pressor substance found normally in blood This question cannot be answered *except by* reference to the hypertension existing in the presence of one ischemic and one normal kidney The hypotheses of best fit *include* both processes retention hypertension developing with azotemia and

has persisted for long often relieves the elevated blood pressure Such experiments *suggests that the ischemic kidney was making something new, a pressor substance* At this point there are two apparently diametrically opposing viewpoints Grollman's experiments show that totally nephrectomized dogs develop chronic hypertension when maintained by peritoneal dialysis or the artificial kidney He therefore believes that healthy kidneys are necessary to maintain normotension (113) In other words the kidney *destroys* normally circulating pressor substances, and on its removal (or in ischemic states) these substances arising elsewhere accumulate The other viewpoint is that the ischemic kidney *makes* pressor substances not normally present from precursors adding something new These two opposing theories can be resolved by moving to a more fundamental level

The kidneys can make vasoactive amines from the proper amino acids Perhaps by decarboxylation, without *deamination vasoactive peptides can be formed* Obviously nephrectomized dogs cannot make these substances In azotemia or *in the absence of the kidneys* however, vasoactive amines are probably formed elsewhere and retained The humoral substances produced by renal ischemia and those accumulating in the absence of the kidneys are different although both are pressor The latter are mainly catechol amines for increased quantities have been found in uremic blood and heart muscle urinary concentrations are low and regitine and benzodioxane lower elevated blood pressure (as in pheochromocytoma) (38) A clinical counterpart is *seen in* cases of azotemic hypertension where the blood *pressure rises only with the blood nonprotein and falls when azotemia* is relieved (Fig 7) Prostatic obstruction is the most common example

TABLE VI

AMINO ACIDS CAPABLE OF FORMING URINARY AMMONIA*

Amino Acid	Renal Amino Acid Oxidase Present	Renal Deaminase Present	(Dog and Rat) Renal Decarboxylase Present	Renal Amine Oxidase Present
Glycine	+			0
L-Alanine	+		+	+
L-Leucine			+	?
L Cysteine			?	?
L Methionine				
L-Aspartic Acid	?		?	?
L Asparagine				
L-Glutamine		+		
L-Histidine			+	+
Oxygen required for enzyme	+	0	0	+

No ammonia formed by glutamic acid lysine or arginine

After Meister (432)

or diamine oxidase which also acts on other diamines such as cadaverine The remainder are oxidized by monamine oxidase

Both of these enzymes are found widespread throughout many tissues. The liver is a rich source Smooth muscle and gut contain them Their ubiquitous nature is all out of proportion to their known metabolic functions

The most interesting aspect of monamine oxidase in reference to renal ischemia is its sensitivity to oxygen lack (Fig 8) Small decrements of oxygen tension inhibit enzymatic activity considerably which is not always the case for other oxidases (89) If this relationship holds in

production hypertension of the ordinary renal or 'es sential variety

How are these often subtle changes made? What are the enzyme systems concerned? Very little is known, but speculation is rewarding

The Amine Oxidase Theory The kidney is an organ of high metabolic activity with one of the largest oxygen consumptions and blood flows of any in the body Filtra tion is a passive process, tubular transport usually an active one The kidney makes some ammonia from glu tamine, thereby providing a base conserving mechanism Other amino acids undoubtedly contribute their nitrogen groups as well, probably by transamination or deamina tion * There are many enzymes in kidney Of them, de carboxylases of certain amino acids have been described of tyrosine, histidine, dihydroxyphenylalanine (DOPA), tryp tophane leucine and 5 hydroxytryptophane Decarboxyla tion is an anaerobic process liberating carbon dioxide from the amino acid and leaving the amine residue

We do not know for certain that amino acid metabolism takes place in the kidney primarily through decarboxyla tion (Table VI) The enzymes are found however, and presumably must act If they do, they can provide bicar bonate for tubular transport Interestingly enough most known decarboxylases are pyridoxal enzymes

The amine residues of these amino acids are the vaso active substances tyramine histamine, dihydroxyphenyl ethylamine, tryptamine isoamylamine and serotonin Histamine is deaminated by a special enzyme histaminase

* There is little or no L amino acid oxidase in mammalian kidney Glycine oxidase is found but for other amino acids to donate ammonia requires either transamination to form glutamine or the two phase reac tion anaerobic decarboxylation and then oxidative deamination by mona mine oxidase

that passed would be distributed to all vascular organs and tissues including brain splanchnic bed and liver In so doing they would be expected to show 1) vasoactivity before being deaminated and 2) some stimulatory or depressant actions on cerebral metabolism

Bacteria in the colon have the proper decarboxylating enzymes for these amino acids and for several others The resultant amines should theoretically act on the vascular system and brain in a similar manner if absorbed That they do not usually so act can be explained by their destruction by amine oxidase in intestinal wall and in liver For it is well known that primary amines and even epinephrine can be ingested in large quantities without systemic effects adding one or more methyl groups to their side chains as in amphetamine or ephedrine however prevents oxidation by hepatic and intestinal amine oxidase allowing the drug to pass unchanged through liver and act on brain or blood vessels No orally active amine vasoconstricting agent lacks this side chain It is possible however that when bacterial flora are selectively inhibited by antibiotics products of intestinal putrefaction can be absorbed into the circulation from the lower colon and cause symptoms especially when the liver is damaged

The fact that extracts of arterial hypertensive blood usually contain more primary amines than those of normotensive blood (90 91) and that certain new or abnormal amines appear in some hypertensive urine (114 115) lends support to the idea that renal amine oxidase is inhibited in most cases of severe hypertension Presumably other renal ischemic states such as shock and congestive heart failure would be associated with the same metabolic abnormality

This attractive theory first propounded by Holtz (87) has received considerable attention from our group

the living animal, we may readily conceive of the consequences of renal oxygen lack

The amino acids decarboxylated by kidney would continue to be so metabolized Oxidation of the amine residues

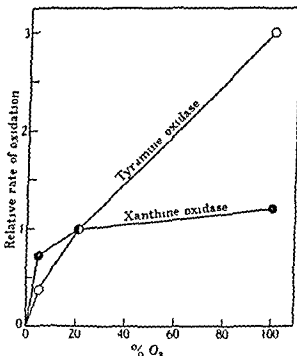


Fig 8 Relative rate of oxidation as a function of oxygen tension Dotted circles are for tyramine or monamine oxidase solid for xanthine oxidase A reduction in oxygen tension of 50 per cent reduces the activity of the enzyme by about 50 per cent. Thus monamine oxidase as opposed to xanthine oxidase is extremely sensitive to oxygen lack (From Kohn H I Tyramine oxidase *Biochem J 31* 1693 1937)

idues would be diminished in proportion to the oxygen lack Presumably these amines would reenter the circulation through the renal vein and be deaminated by monamine oxidase at other sites where there is adequate oxygen First, the lung would take some of them out, those

amines (which is theoretically possible) a minor vicious circle could be induced The result would be minor degrees of fluctuating neurogenic hypertension and many of the symptoms of the neurogenic hypertensive state

Since we are concerned primarily with the killing factor in hypertension and not with mild neurogenic vasospastic states it is well to examine the properties of this enzyme system further There are two which are of help in implicating a disturbance of amine oxidation First of all amine oxidase acts on hypertensin or angiotonin (4 112) This pressor amine is a complex polypeptide (119) Second the enzyme acts on pherentasin This pressor amine is probably a polypeptide (120) If the enzyme can act on terminal amines of peptides it is possible that the formation of such peptidic amines occurs through decarboxylation and destruction by terminal amine oxidation Therefore we cannot exclude monamine oxidase in any theory of pathogenesis

This enzyme probably needs vanadium as a cofactor Vanadium occurs in three valence states and is therefore a good metal for oxidation reduction reactions being used as such by certain ascidia which concentrate it from sea water While not shown to be an essential trace element for man vanadium is found in tissues of mammals and occupies a place in the periodic table where essentiality might be inferred This subject will be discussed further in Chapter VI

Possible Role of the Lungs In order to cover other conceivable mechanisms of vasospasm induced by humoral pressor substances we cannot *neglect the* pulmonary circulation Any vasoactive material formed in an organ and discharged into the venous circulation must pass through the lungs before entering the area of action the peripheral arterial bed The lungs destroy at least one vasoactive

Partly purified but still crude amine oxidase, injected into rats, *prevents the* pressor action of both renin and pherentasin (*vide infra*). Furthermore, hypertensin or angiotonin is a good substrate for the enzyme (112, 116) indicating the presence of a primary amine group necessary for activity, for the reaction mixture is vascularly inert or depressor. Renal hypertensive dogs can be maintained normotensive on daily injections of the active enzyme (117, 112). All naturally occurring pressor substances are amines. Why, then, does not this enzymatic disturbance account fully for the establishment and maintenance of hypertension?

Perhaps it does, but not through the mediation of the substances listed. For all of them are relatively weak pressor amines when compared to norepinephrine. Very large quantities would be required to cause hypertension, amounts readily detectable in blood. Furthermore, a mixture of these amines would be expected to produce a peripheral circulatory profile different from that seen in hypertension, in so far as is known, for many of them have selective actions on different vascular beds (112), although all, including histamine, constrict the vessels of the kidneys (118). Therefore, circulating primary amines from simple amino acids cannot be implicated as direct causes of generalized vasospasm. They can be concerned, however, with some of the minor manifestations of hypertension, such as headaches, anxiety, tension, nervousness, insomnia, the diencephalic blush, flushing, sweating and the like. If they can cause nervous and emotional tension (which some of them can), and if nervous tension can cause peripheral vasoconstriction through sympathetic nervous discharges (which it can), and if sympathetic discharges can cause neurogenic renal ischemia (which it can), and if renal ischemia can produce circulating primary

ably is associated with renal abnormalities (90 91 120 124) As far as is known it is the only pressor substance found so far in hypertensive blood but not in normotensive blood There is more in arterial than in venous blood

Pherentasin has a prolonged pressor action in rats especially those with renal hypertension (91) It also constricts the smooth muscle of the isolated rabbit aorta (120) Because of its strong pressor action and the small quantities present isolation and identification has been most difficult. Much of what is known of its nature comes from inactivation studies by known agents

Pherentasin probably contains a trace metal essential for activity the nature of which is unknown It is inactivated by many antihypertensive agents not acting on sympathetic nerves (Table VII) and disappears *from the* blood when hypertension is controlled No one knows how it is formed.

Renin Now largely discarded the mechanism for the formation of renin by ischemic kidneys supplied an attractive hypothesis to explain chronic renal hypertension When the kidney is made ischemic renin is released into renal venous blood from parenchymal tissues possibly the juxta-glomerular apparatus (125) Thus proteolytic enzyme acts on an α_2-globulin made in liver partially hydrolyzing it to hypertensin or angiotonin * a vasoactive polypeptide As with most other more simple substances this pressor amine does not produce the peripheral circulatory profile of the hypertensive state as does norepinephrine (126 127) Unfortunately for the theory renin and its effector substances have not been found in renal venous blood of dogs or human beings with chronic hypertension They do appear however in the acute vasospastic states of acute

* Hypertensin and angiotonin are used interchangeably except when reference is made to a specific preparation One term should be dropped.

material, serotonin, and probably others, for over a hundred years physiologists have taken advantage of this property to free shed blood of "spatgift" and "fruhgift" constrictor and dilator substances in preparation for perfusion experiments Presumably monamine oxidase is the enzyme which deaminates constrictor primary amines in the pulmonary circuit

Let us for a moment consider what might happen if all pulmonary monamine oxidase were inhibited A portion of the pressor amines formed normally by kidney or tissues would be transported unchanged from venous to arterial circulations and would act on the peripheral blood vessels, the blood pressure would rise unless cardiac output were depressed The remainder would constrict the pulmonary vascular bed, causing pulmonary hypertension If *extra* quantities of primary amines were formed by kidney (or liver) and pulmonary monamine oxidase were inhibited or saturated beyond its capacity to oxidize them, further vasoconstriction would result Therefore, the pulmonary circulation could play a part in hypertension

In this respect it is interesting that the lungs were the only organs in which Tipton detected vanadium the possible cofactor of monamine oxidase The pulmonary circulation responds to hypoxia by constriction The lungs contain many abnormal trace elements notably aluminum and titanium, a known enzyme inhibitor This hypothesis to our knowledge has not been explored

SPECIFIC EFFECTOR SUBSTANCES

Pherentasin Although pherentasin has never been proven to come only from the kidney, this pressor amine of probable peptide nature is found in increasing quantities in the blood of patients with severe hypertension, is difficult or impossible to detect in mild stages has been obtained from the renal vein of two patients and presum

TABLE VIII

COMPARISON OF PROPERTIES OF ANIMAL HYPERTENSIN AND HUMAN PHERENTASIN

Inactivation by	*Hypertensin* (*1*)	*Pherentasin* (*2*)	*Method and Remarks*
Drying	0	+	(1) can be lyophilized
Heat at pH 8.8	+	+	
Heat at pH 2.0	0	0	
Nitrous acid	+?	+	
Ninhydrin	+?	+	
Semicarbazide	?	±	(2) alters to a rapid reactant
Hydroxylamine	?	±	(2) alters to a rapid reactant
Amine Oxidase	+	+	
Tyrosinase	+	0	
Papain+cysteine	+?	+	
Chymotrypsin	+	0	
Carboxypeptidase	+	0	
Trypsin	+	0	
Pepsin	+	0	
Mg^{++}	0	0	
Mn^{++}	+	+	(1) rapid (2) slow
Cr^{++}	?	0	
Co^{++}	+	±	(1) rapid (2) partial
Fe^{++}	0	0	
V^{++++}	–	–	Both enhanced
Cu^{++}	0	0	
Zn^{++}	0	0	
Hydralazine	+	+	(1) more sensitive
NaSCN	+	+	
8-Hydroxyquinoline	+	+	(1) slow (2) more rapid
EDTA Na_2H_2	0	±	(1) 50% in 22 hours (2) 50–100% in 3–6 hours
NaN_3	+	+	
$Na_2Fe(CN)_5NO$	+	+	Rapid for both
1 benzyl 2 methyl 5 methoxy tryptamine	+	+	Serotonin antagonist

NOTE While distinct differences between these two substances are obvious the hypertensin used was probably principally hypertensin I (angiotonin) obtained from hog renin and serum Pherentasin may be hypertensin II of human origin with a slightly different structure since there is no reason to believe that the α_2 globulins of pig and man are identical

TABLE VII

INACTIVATION OF PHERENTASIN BY METAL IONS AND METAL BINDING AGENTS (120)

Estimated Activity Per Cent of Control Values

Substance	*Immediate*	*4–5 hr*	*24 hr*	*Boiled after 24 hr*	*Color Developed*
Mg^{++}	91	130	61	0	0
Cr^{++}	187	180	*12*	0	0
Mn^{++}	*45*	*17*	0	—	Pink cloudy
Fe^{++}	70	134	58	0	0
Co^{++}	59	50	50	0	Faint pink
Cu^{++}	96	107	*27*	0	0
Zn^{++}	84	137	54	27	0
Hg^{++}	168	51	115	0	0
None	100	100	100	80	0
Hydralazine	*46*	*20* (2)*	0 (18)		Deep blue
NaSCN	110	0 (1½)			Yellow-orange
$Na_2Fe(CN)_5NO$	110	0 (2)			0
NaN_3	89	0 (2)			0
8 Hydroxyquinoline	*35*	0 (1)			Pale green
Na_2H_2EDTA	100	0 (3)			0
Cysteine	100		100 (120)		0

All metals were added to the active material in 0 01 M concentrations giving a final concentration in the 20 ml bath of 0 0005 M The binding agents were added in 2–5 mg amounts per ml extract None of the metal ions alone affected the test system at 0 0005 M concentrations. The test method was that of Furchgott (85)

Figures in italics represent more than 50% inhibition

* The figures in parentheses indicate the number of hours of incubation at room temperature when different from that shown at the top of the column

renal ischemia toxemia of pregnancy, shock acute nephritis and congestive heart failure (128 131)

The validity of the renin mechanism is unquestioned as a defense reaction to acute and sub acute circulatory changes When these become chronic renin is replaced by another vasospastic mechanism, probably that of pherentasin

Pherentasin may be a form of hypertensin for there are similarities between the two substances There are also dissimilarities The known properties of the two are listed in Table VIII It is possible that metabolic alterations

animals to normal levels (132) It is not renin As a protein it probably acts enzymatically Possibly sustained pressor principle is a precursor of pherentasin or represents another renal pressor mechanism

Vasoexcitor Material This unidentified substance has the property of sensitizing blood vessels to epinephrine when the latter is topically applied It comes from ischemic kidney and is active in minute amounts Larger quantities appear in chronic hypertension and congestive heart failure (100) Other substances such as renin pherentasin sustained pressor principle and some primary amines also have this property which may be nonspecific

Others A great many vasoactive substances have been found in urine and blood most of them eventually showing up as primary amines or more complex structures They may represent metabolic by products of the basic renal abnormality No good case for any has been proven as directly concerned in chronic generalized vasospasm (133-135 112 4)

Comment When many different substances are discovered or suspected to cause a single recognized abnormality time usually leads to the abandonment of all but one as causative factors Let us attempt to gather all of these different substances together and fit them into one unified scheme To do so we must speculate

1 Renin may be involved in experimental renal hypertension The evidence for this statement is indirect in that anti renin prepared by immunization reduces the hypertension of renal hypertensive dogs (136) Anti hog renin neutralizes hog and dog renin and canine hypertension anti monkey renin neutralizes monkey and human renin and simian hypertension (137) These anti renins are therefore species specific to some degree

2 If anti monkey renin is found to affect human hyper

occurring with time form the former from the latter, or that both have a common precursor Hypertensin appears to require a metal as an activator

Posterior Pituitary Factor A pressor substance of polypeptide nature which has received some serious attention in hypertension is vasopressin While a smooth muscle stimulant in small doses, it also has such antidiuretic properties that little speculation concerning its role in hypertension has been aroused The direct relationship of the hypothalamus and the stalk of the pituitary, the known but minor electrolyte imbalances found and the peptide nature of the substance make it not inconceivable as an effector substance, provided some minor alteration in its molecule negates its antidiuretic properties While not a nephrogenic substance one of its actions is on the kidney, if pressor, its release by the pituitary might be expected to cause widespread vascular constriction The antidiuretic dose, however, is very small compared to the pressor dose In our experience even large amounts do not constrict the smooth muscle of the isolated rabbit aorta, as pherentasin and hypertensin do Although it is possible that pherentasin may be a renal metabolic product of vasopressin, there is no proof or disproof of this idea Interestingly enough, however, pitressin has been used to treat hypertension, with most variable and inconclusive results *

Sustained Pressor Principle A protein obtained from ischemic renal tissue or blood of animals in shock has the property of restoring the low blood pressure of pithed

* Pitressin or vasopressin may require an activator for commercial preparations are inactive on the rabbit aortic strip It exists in a ring form with an S-S linkage (435) We have attempted to activate it by adding copper cobalt ferrous iron zinc manganese nickel and mercury without success save for an equivocable slight activation with copper Oxytocin or pituitrin is also inactive in this system

contain a terminal amine group necessary for activity Both can act as a VEM Renin is inactivated *in vivo* by crude monamine oxidase (112)

6 Alterations may occur by two mechanisms decarboxylation of a terminal carboxyl leaving a peptide amine or preferably peptide splitting leaving a terminal amine

7 The substrates as well as the renins from different species are obviously different in composition Human and primate renin will react with the substrates from all mammals tested while animal renin will not react with α_2-globulins from primates The exhibition of pressor activity of all hypertensins does not in any way mean that they are identical in chemical composition but only that they have in common an active group probably a terminal primary amine Pepsin acting on casein produces a pressor peptide pepsitensin identical in action to hypertensin There are thus many variables in species the source of a renin the source of serum α-globulins which almost certainly differ in composition from one species of animal to another and perhaps the nature of the plasma enzyme converting the inactive peptide into its vasoactive form The different amino acids found by various workers can be perhaps explained by the different sources from which the renin and its substrate were obtained (Table IX) Since human renin is unique to primates and since human globulins are unique to man human hypertensin can be expected to be unique in its composition of amino acids Therefore human hypertensin may have only a moderate resemblance to that obtained from horses dogs pigs and cows and could well be pherentasin

8 There is no good evidence that renin or pepsin break down their protein substrates into substances having a terminal primary amine Activation most likely occurs

tension, one can assume that renin or some similar protein is involved in human nephrogenic hypertension But renin is found in renal venous blood only in acute vasospastic states and not in chronic hypertension, either experimental or clinical Therefore, it must remain in the kidney, a highly speculative point

3 Hypertensin or angiotonin is found only in acute vasospastic states It has two forms, hypertensin I, inactive on isolated smooth muscle but active in blood,* and hypertensin II, a much more highly constrictor and pressor substance (138, 139) An enzyme in plasma converts I to II (141) apparently this is a metalloenzyme, requiring chloride and another metal which is tightly bound Perhaps this enzyme is, or acts like, sustained pressor principle

4 Pherentasin is found only in chronic vasospastic states Perhaps pherentasin is a form of hypertensin or angiotonin, altered either by a slightly changed renin by a slightly different protein substrate, or by a new enzyme developing in chronic vasospasm, such as a peptide decarboxylase attacking the terminal carboxyl group

5 Both of these substances are peptides inactivated by metal binding antihypertensive drugs and *therefore* probably contain a metal necessary for activity Both are inactivated by monamine oxidase and therefore probably

* Hypertensin I obtained from the action of hog renin on horse serum contains the single amino acids aspartic proline valine isoleucine leucine tyrosine phenylalanine arginine and two molecules of histidine (139) Peart using hypertensin from rabbit renin and beef serum disagrees slightly in that there was no isoleucine and two molecules of valine (142) Obviously tyrosinase inactivates both through the tyrosine portion of the molecule amine oxidase attacks the terminal primary amine probably on aspartic acid Manganese inactivates by pseudopeptidase activity (140) The sequence of amino acids in Peart s hypertensin is Asp Arg Val Tyr Val His Pro Phe His Leu (142b)

through a manganous peptidase (295) Thus, we can postulate several theoretical alternate reactions based on all the evidence (Fig 9 the active materials are in italics)

Reaction A would thus occur in acute vasospastic states while reactions B C or D might take place in chronic states Pherentasin needs no serum for activity that it has at least six amino acids is based upon the findings of the active material showing six spots in chromatograms A more unitary hypothesis is that pherentasin is actually hypertensin II a matter on which we have no evidence as yet

Comment The pieces of the picture puzzle are falling into place but there is need for much work to be done before the outlines are clear

Locus of Action of Pressor Substances While not directly established it is reasonable to assume that all vascular smooth muscle is constricted by *the pressor sub-*

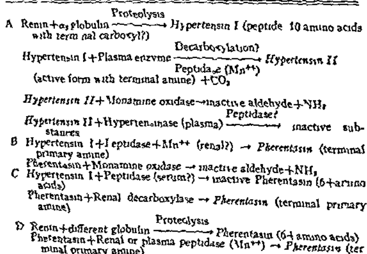


Fig 9

TABLE IX

AMINO ACIDS IN VASOACTIVE PEPTIDES

Amino Acid	Hypertensin (142)*	Hypertensin (139)†	Hypertensin (436)	Vasopressin (435)	Oxytocin (435)	Pepsitensin (434)	Common to All
Histidine	2	2	+				
Arginine	1	1		1		+	
Aspartic acid	1	1	+	1	1	+	Aspartic acid
Proline	1	1	+	1	1	+	Proline
Valine	2	1	+			+	
Lysine			+				
Leucine	1	1	+		1	+	Leucine
Isoleucine		1			1		
Phenylalanine	1	1		1		+	
Tyrosine	1	1	+	1	1	+	Tyrosine
Alanine			+			+	
Serine			+			+	
Glutamic acid			+	1	1	+	
Glycine			+	1	1		
Threonine							
Cystine				2	2		
Methionine							
No amino acids	10	10	11	9	9	10+?	

NOTE The differences in hypertensin may be due to the sources of the α_2 globulins from different species hog beef and horse Vasopressin from hogs differs from that of beef in that leucine replaces arginine (435) Pepsitensin was obtained from casein (434)

* Rabbit renin+beef serum

† Hog renin+horse serum

to do more than detect the grosser lesions A disease causing renal ischemia which then influences hypertension is often unsuspected because the cardiovascular manifestations of the elevated blood pressure may mask the underlying renal abnormality

Organic Parenchymal Renal Disease The most common diseases of the kidney producing ischemia are pyelonephritis and glomerulonephritis The former is often a low grade smouldering fibrosing disorder without systemic manifestations and often without obvious urinary changes (143) The latter may be masked insofar as the urinary sediment is concerned by the superimposed hypertension To list the other more unusual renal diseases congenital or acquired is hardly within the province of this discussion most are often but not always associated with hypertension (144 4)

Organic Extra renal Arterial Disease Atherosclerosis of the mouths of the renal arteries is common in generalized and in aortic atherosclerosis The mechanism for the deposition of lipid in plaques about the orifices of bifurcating arteries is not known Undoubtedly pressure changes play a part possibly the presence of increased numbers of vasa vasorum at such bifurcations influence the lesions Therefore when atherosclerosis involves the renal arteries partial renal ischemia may result with subsequent elevation of the blood pressure in predisposed individuals With aortography becoming more common such lesions are more frequently demonstrated According to Blackman they are the usual findings in hypertensive patients (145) It is possible that they represent the most common form of the disease in individuals beginning to be hypertensive over the age of 50 They are difficult to demonstrate at necropsy (146 147)

Because the existence of these lesions has not been

stance (or substances) responsible for the humoral component of sustained hypertension If this is so, the following physiological alterations in vascular volume can be expected

1 An increase in aortic volume, for the aorta is predominantly an elastic and not a muscular organ As elastic limits are approached with increasing pressures the rate of increase of volume lessens

2 A *decrease in the volume of blood in muscular arteries*

3 If veins also took part in the process, venous volume in the smaller muscular veins should be decreased without, however change in central venous pressure

It is often difficult to detect the high pulse pressure in hypertension by feeling the dorsalis pedis arteries or even the radials When blood pressure is lowered by hydralazine, the pulses in these smaller arteries become full Thus, as pulse pressure falls the detectable pulsations in muscular arteries increase This seeming paradox is easily explicable on the basis that the muscles of these arteries are constricted in hypertension and that their volumes are diminished

The high pulse pressure seen in most patients with hypertension is probably due to a relative loss of aortic elasticity because of stretching under pressure Thus, the aorta becomes physiologically hardened ' In children and young adults we see low pulse pressures with diastolic hypertension probably because their aortas are more elastic than are those of older people

ANATOMICAL CAUSES OF RENAL ISCHEMIA

A variety of mechanisms and lesions can account for renal ischemia in man, many with experimental counterparts *Unfortunately our diagnostic methods are too crude*

in the two groups Yuile (158) recently reviewed the literature on the relation of obstructive lesions of the main renal artery and hypertension and concluded that such a relationship does exist in certain cases This author pointed out the desirability of closer anatomical and physiologic correlation

Organic Intra renal Arterial and Arteriolar Disease The almost universal lesion found in the kidneys of patients with hypertension at necropsy is renal arterial and arteriolar sclerosis This lesion is not the cause of the hypertension however but is the result And a late result at that. About 50 per cent of patients having renal biopsies done during the operation of lumbodorsal sympathectomy had little or no arterial or arteriolar sclerosis (159) This lesion has been shown to result from hypertension produced by a variety of causes in rats (160 163) rabbits (164) and dogs (165 7) Serial renal biopsies in dogs over a seven year period have demonstrated the gradual development of the lesions only after 2 to 4 years of both neurogenic and unilateral renal hypertension the first sign being a thickening of the glomerular capsule and later an increase in material in the glomerular tuft staining with periodic acid (7)

None of these renal diseases alone can be said to cause hypertension in man until azotemia develops Hypertension is absent in 30 to 50 per cent of patients with the first two types in non azotemic stages The third type of course is the result of hypertension They do however influence it profoundly and may often alter its course to a progressive and severe one That quality which we call the ability to react to stress by vasospasm must apparently be present first and in conjunction in order for severe sustained hypertension to develop in patients with organic renal ischemia

Why then are there no more cures of hypertension

emphasized in the recent literature, we quote from Braun Menendez *et al* in 1946 (148) "Goldblatt (149) was the first to show that hypertension was associated in some cases with sclerosis and narrowing of the orifice or of the lumen of the main renal artery Leiter (150) somewhat later described a case of chronic hypertension associated with complete arteriosclerotic occlusion of the left renal artery and incomplete occlusion of the right Freeman and Hartley (151) almost simultaneously reported hypertension in a patient who was nephrectomized because of an accident At autopsy an atheromatous plaque was found to obstruct the mouth of the renal artery Similar cases were described later by Blackman (145), Stewart (152) Saphir and Ballinger (153) and Laas (154) The importance of unilateral narrowing has been emphasized by Oppenheimer, Klemperer and Moschkowitz (146) who showed that in 18 cases who anatomically showed unilateral narrowing of the renal artery, 15 had hypertension Blackman (145) found a narrowing of the renal artery at or near its mouth in 86 per cent of cases with hypertension Richardson (155) recently reported stenosis of one or both renal arteries by arteriosclerotic plaques in 25 of 32 hypertensive patients studied at autopsy

Kahn and Laipply (147) *observed a high incidence of* bilateral arteriosclerosis in 1 000 hypertensive patients studied pathologically Friedman Moschkowitz and Marrus (156) observed arteriosclerosis of the renal vessels in 23 of 28 hypertensive patients who were nephrectomized

Lisa, Eckstein and Solomon (157) reported that in 100 consecutive cases coming to autopsy in which blood pressure readings were available, hypertension was present in 56 while 44 were nonhypertensive No appreciable difference in the average diameter of the renal artery was found

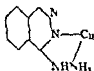
matter of fact many effective drugs bind metals in one way or another (Chapter VI) This common property immediately focuses attention on metalloenzymes in kidney and vascular smooth muscle It also stimulates considerable thought about the role of trace metals in pathogenesis of severe hypertension in which the neurogenic component has become of minor consequence

The agents used in man are hydralazine and its derivatives thiocyanate ion sodium nitroprusside 2 3-dimer captopropanol (BAL) sodium azide and ethylenediamine tetra acetate Of practical interest for continuous use are only the first three the effects of the other three being short lived (Table X)

HYDRALAZINE AND OTHER CHELATING AGENTS

Hydralazine and its derivatives are unique drugs No other agents known produce the same actions on vascular smooth muscle Understanding of their mode of action is the key to understanding of pathogenesis and possibly etiology of severe hypertension While imperfectly understood a consideration of their pharmacological chemical and enzymatic actions is necessary

Chemical Reactions Hydralazine like other hydrazides is a strong chelating agent. It will form a complex with iron copper tin vanadium, manganese nickel silver and mercury The possible structure is



making a five sided ring with nitrogen a most stable chelate This property is shared by isonicotinic acid hydrazide (isoniazid) and probably its isopropyl derivative

in cases of unilateral renal diseases subjected to nephrectomy? The answer is obvious If hypertension once long established, can cause bilateral renal arteriolar sclerosis removal of the one primarily affected kidney will not remove *all* of the ischemic renal tissue On the other hand, nephrectomy done in time may result in temporary or semi permanent cure (166, 167) Experimental counterparts of this situation are known in rabbits, which get permanent hypertension after removal of an ischemic kidney which has been in place for three months or more (164), two of our unilateral renal ischemic dogs suffered autonephrectomy, without influencing their long established hypertension

Comment These three types of organic renal disease can be considered as accessory factors in pathogenesis but not primary ones They probably do not cause hypertension in themselves without the neurogenic factor being present

DRUGS ACTING ON NEPHROGENIC MECHANISMS

We can learn something about nephrogenic mechanisms from the actions of specific drugs, although the effective agents are few and have several actions In experimental hypertension however, there are broader leads Three types of agents are active metal binding agents hydralazine and some other hydrazides (also metal binding agents) and pyrogens The latter apparently dilate the renal vascular bed in some unknown manner allowing greater blood flow and therefore counteracting renal ischemia

Metal Binding Agents All of the antihypertensive drugs used in man which do not apparently affect neurogenic pathways, have in common the ability to bind trace metals There are no known exceptions to this statement As a

(iproniazid) whose pyridine bases in themselves weakly bind metals without the hydrazide group (Table XI) Distinct specificities for metals are exhibited however

Hydralazine is also a carbonyl reagent as are some other hydrazides phenyl hydrazine for example which forms an ozozone with glucose It will bind pyruvate acetate and acetaldehyde (168) Hydralazine has specific reac tions in that no ozozone is formed with glucose or lactic acid It does not combine with any of the steroids tested (168) It is 1 hydrazinophthalazine (Apresoline)

This agent also complexes with the sulfhydryl groups on cysteine glutathione 2 3-dimercaptopropanol (BAL) and other simple mercaptans The complex can be dissociated readily by arsenic

TABLE XI A

Isoniazid * Hydralazine and Metals

	Isoniazid			
	Destruction by Me^{++} Auto-claving %	*Destruction by Me^{++} H_2O_2* %	*Binding of Hydralazine*† $+Me^{++}$ %	*Similarities*
Mg^{++}	0	5	0	+
Ca^{++}	0	5	0	+
Mn^{++}	100	100	87	+ Greatest at pH 6 5–7 0
Fe^{++}	50	80	22	
Fe^{+++}	40	95	100	+
Co^{++}	40	35	0	
Ni^{++}	10	15	48	
Cu^{++}	100	100	100	+ Greatest at pH 9.5–10 0
Zn^{++}	15	15	0	+

* Lewin E and Hirsch J G Studies on the stability of isoniazid. *Am Rev Tuberc & Pulm Dis* *71* 732 1955

† Perry H M Jr and Schroeder H A Studies on the control of hypertension by Hyphex III Pharmacological and chemical observations on 1 hydrazinophthalazine *Am J M Sc* *228* 396 1954

TABLE X

SUBSTANCES WITH METAL BINDING PROPERTIES SELECTIVELY AFFECTING ARTERIAL HYPERTENSION (312)

Substance	*Rat*	*Dog*	*Man*	*Metals Bound*	*Reference*	*Remarks*
Thiocyanate		+	+	Many	173	Used in industry CuSCN insoluble
Nitroprusside	+	+	+	Many	174	Zinc Reagent
Azide*	+	+	±	Many	175	Reactant
2 3-dimercaptopropanol (BAL)*	+	+	+	See Text	182	Chelator
Hydralazine and other hydrazines	+	+	+	See Text	168	Reactant
Tetrasodium pyrophosphate†	±			Many	4	Detergent and Reactant
9 mercaptans**	+	+ (2)		Many	182	Sulfhydryl binding
6 sulfur compounds	+			Several	182	SCNH most active
8-hydroxyquinoline	+			Many	183	Chelator
Perma Kleer	+				183	Polyamino carboxylic resin
Ca^{++} EDTA	+		±	Many	180 183	Slight effect in man
Cr^{++} EDTA	+				183	But not chelates of Fe^{++} Zn^{++} Ni^{++} Cu^{++}, Fe^{+++}
Mn^{++} EDTA	+				183	
Co^{++} EDTA	+				183	

* Short acting

† Large doses Only active phosphate of 11 tested

** Ten others inactive 5 nonspecific

TABLE XII

INHIBITION OF HISTAMINASE BY HYDRAZIDES

Substance	*Concentration Producing 50% Inhibition (molar)*	*Antihypertensive Effect*
Guanidine HCl	10^{-3}	0
Thiosemicarbazide	10^{-6}	+
Semicarbazide HCl	5×10^{-6}	?
Hydrazine SO_4	8×10^{-7}	?
Aminoguanidine HCO_3	5×10^{-6}	0
1-4 dihydrazinophthalazine	$2\ 3\times10^{-6}$	+
1 hydrazino-4 methylphthalazine	$2\ 5\times10^{-6}$	+
1 hydrazinophthalazine	6×10^{-6}	+

Gross F Schuler W Tripod J and Meier R Inhibition of diaminoxidase (histaminase) by phthalazine derivatives *Experentia* 8 229 1952

Schuler W Inhibition of diaminoxidase (histaminase) *Experentia* 8 230 1952

It binds strongly to arterial mash serum proteins egg albumin and some polypeptides possibly through carbonyl or sulfhydryl linkages It does not bind with casein nor with mixed amino acids

Enzymatic Reactions Hydralazine is also an anti-enzyme for several known systems It and its derivatives are strong antihistaminases theoretically preventing histamine formed from histidine from being destroyed rapidly but not necessarily causing release of histamine from histidine (Table XII) Histamine can come from the action of histidine decarboxylase believed to be a pyridoxal enzyme if so inhibition by hydralazine might be suspected Hydralazine is a potent inhibitor of DOPA decarboxylase in small concentrations also a vitamin B_6 enzyme (Table XIII) There is some evidence that histaminase itself may be a pyridoxal enzyme (169)

TABLE XI B

EFFECT OF METALS AND BINDING AGENTS ON HISTAMINASE (437)

(Substrate Cadaverine)

Inhibition of Reaction %

Concentration	*No Drug*			*Hydralazine*			*Isoniazid*			*Ba 12 630*		
	10^{-3}	10^{-4}	10^{-5}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-6}
Mn^{++}	8	0		0				50		0		
Fe^{+++}		0		0			0			0		
Co^{++}	0				50		0					50
Ni^{++}	0	0		50			20				50	
Cu^{++}		25	7	50				0		33		

Schuler W and Meier R Releasing action of metals on the hydrazine inhibited enzymatic oxidation of cadaverine *Arch Exper Path* *223* 169 1954

NOTE The enzyme is inhibited by hydralazine isoniazid and Ba 12 630 2 4-di hydrazinoquinazoline The metals alone had little effect on the enzyme but inhibition of the enzymatic reaction by both hydralazine and Ba 12 630 was prevented by Co^{++} and Ni^{++} that by hydralazine by Cu^{++} that by isoniazid only by Mn^{++} and none by Fe^{+++} The reaction between drug and enzyme was irreversible once it had occurred These results offer indirect evidence for metal binding capacities of the three agents although histaminase (diamine oxidase) is not known to require a metal for activity

TABLE XIII—(*continued*)

Substance	*DOPA Decarboxylase Millimolarity of agent*				*Monamine Oxidase (Substrate tryptamine) Millimolarity of agent*			
	10	*1*	*0 1*	*0 01*	*10*	*1*	*0 1*	*0 01*
Isoniazid	41	84	99	100	75	98	103	101
Iproniazid	108	—	—	—	14	*52*	91	101
Pyridoxal Isoniazid	100	114	92	91 (90)	96	101	101	101
1 5 diphenyl 3 thiocarbo-hydrazide	100	97	—	—	—	—	—	—
8 hydroxyquinoline sulfonic acid	102	—	—	—	105	—	—	—
Reserpine	102	90	92	94	—	—	115	—
β Mercaptopropionic acid	81	98	100	97	123	111	103	95
Tetrasodium pyrophosphate	86	101	97	—	95	—	—	—
Sodium cyanide	66	85	98	97	45	98	95	97
Sodium thiocyanate	89	98	100	110	100	—	—	—
Sodium azide*	91	94	—	—	115	116	98	94
Choline azide	100	—	—	—	112	104	101	101
Sodium Nitroprusside*	63	*71*	*76*	91	129	98	101	100

Italicized figures represent 20% change at 1 0 millimolar concentrations or le s considered significant

Those in parentheses show further dilutions by 10

* Antihypertensive in man Note that some of these tend to depress one enzyme and enhance the other

TABLE XIII

EFFECT OF METAL-BINDING AND ANTIHYPERTENSIVE AGENTS UPON TWO RENAL ENZYME SYSTEMS (GUINEA PIG)

(% *Activity*)

Substance	*DOPA Decarboxylase Millimolarity of agent*				*Monamine Oxidase (Substrate tryptamine) Millimolarity of agent*			
	10	*1*	*0.1*	*0.01*	*10*	*1*	*0.1*	*0.01*
1 hydrazinophthalazine C 5968* (Hydralazine)	12	*23*	*59*	88 (99)	163	*159*	118	103
1-4 dihydrazinophthalazine C 7441* (Nepresol)	5	*21*	*21*	*66* (83) (100)	144	*156*	118	103
3 hydrazine 6-phenyl diazine C 6084*	4	*39*	*74*	87 (89) (90)	87	118	95	93
1 4 dihydrazinopyridazine C 13504*	20	*33*	*61*	102	25	82	98	84
Phthalazine C-7182	87	—	99	98	61	103	101	96
1 hydrazino isoquinoline HCl C 7406	20	*42*	*76*	97	49	103	98	102

TABLE XIV B

EFFECT OF ADMINISTRATION OF HYDRALAZINE ON URINARY EXCRETION OF 4-PYRIDOXIC ACID (MG) (111)

	No Subjects	No Tests	Mean Before	N Tests	Mean During	Dose of Hydralazine
Begun on Hydralazine	4	9	24 5 *(17 3–33 5)*	18	15 5 *(11 2 23 2)*	150-600
On Hydralazine for 1–3 Years	6			28	12 8 *(5 5–18 7)*	200-600
O EDTA†	3	10	22 1	15	24 0 *(22 0–26 0)*	
On EDTA and hydralazine	4			22	12 7 *(4 4–18 8)*	200-600
Normal	5	22	21 8			
Atherosclerosis	4	22	27 4			

Per 4 hours after 0 mg. orally I pyridoxal hydrochloride
† Calcium d sod um ethylenediamine tetraacetate intravenously
The ranges for each group shown in italics are the mean excretion rates of each patient

chelate trace metals The antituberculous hydrazides are not antihypertensive however with the possible exception of isoniazid The key to antihypertensive activity lies in the specificity of the hydralazines for a reaction not exhibited by other hydrazides and similar agents

The known actions of hydralazine are listed in Table XV The actions of a large number of similar substances are shown in Table XIII as regards the two enzyme systems considered here

Other Metal Binding Agents Thiocyanate ion is used in industry for making soluble salts of a number of metals In man according to Sollmann (173) it hastened the elimination of metals perhaps by rendering the metal protein compounds more soluble In Table XVI is a partial list of the soluble metallic salts of thiocyanate Symptoms

Whether or not hydralazine causes excretion or deficiency of vitamin B_6 is not known It does seem to interfere with the conversion of pyridoxal to its metabolite 4 pyridoxic acid (Table XIV) A relative, isoniazid (isonicotinic acid hydrazide), promotes the excretion of a pyridoxal isoniazid complex in urine and can cause peripheral neuritis in patients taking large amounts for tuberculosis presumably the neuritis is due to vitamin B_6 deficiency (170) Isoniazid is a good inhibitor of DOPA *decarboxylase* and a poor one of monamine oxidase (Table XIII) Its derivative iproniazid (isonicotinic isopropyl hydrazide) *does not affect DOPA decarboxylase but is a strong inhibitor* of monamine oxidase Both inhibit histaminase (171, 172) The latter cannot be used clinically because of its 'benzedrine like reactions of euphoria and *cerebral* stimulation, believed to be due to cerebral and peripheral inhibition of this oxidase, thus allowing natural primary amines to circulate

We are discussing these related hydrazides because of their similarities of structure their antienzymatic activities *their affinities for vitamin* B_6 and their abilities to

TABLE XIV A

EFFECT OF REPEATED ADMINISTRATION OF PYRIDOXAL HYDROCHLORIDE UPON EXCRETION OF 4 PYRIDOXIC ACID (MG) (111)

Subjects	*No*	*Mean Total Dose Vitamin B_6 (mg)*	*Mean Days Given*	*Amount Excreted on Last Test*	
				Mean	*Range*
Normal	5	250	5	22 3	9 8–27 8
Atherosclerosis	4	250	5	27 8	21 8–32 4
Patients on EDTA†	3	1470	18	21 0	18 0–24 2
Patients on Hydralazine	11	700	12	11 3	2 6–20 5

TABLE XIV B

EFFECT OF ADMINISTRATION OF HYDRALAZINE ON URINARY EXCRETION OF 4 PYRIDOXIC ACID (MG) (111)

	No Subjects	*No Tests*	*Mean Before*	*No Tests*	*Mean During*	*Dose of Hydralazine*
Begun on Hydralazine	4	9	24 5 (*17 3–33 5*)	18	15 5 (*11 2 23 2*)	150–600
On Hydralazine fo 1 3 Years	6			28	12 8 (*5 5 18 7*)	200–600
On EDTA†	5	10	22 1	15	24 0 (*2 0–26 0*)	
On EDTA and hydral azine	4			22	12 7 (*4 4–18 8*)	200–600
Normal	5	22	21 8			
Atherosclerosis	4	22	27 4			

* Per 4 hours after 50 mg orally of pyridoxal hyd ochloride.
† Calcium d sodi m ethylenediamine tetraacetate intravenously
The ranges for each group hown in italics are the mean excretion rates of each patient

chelate trace metals The antituberculous hydrazides are not antihypertensive however with the possible exception of isoniazid The key to antihypertensive activity lies in the specificity of the hydralazines for a reaction not exhibited by other hydrazides and similar agents

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TABLE XV

SUMMARY OF PROPERTIES OF HYDRALAZINE OTHER THAN CARDIOVASCULAR

Basic Chemical Reactions In Vitro	*Reference*
Metal binding	
complete for Fe^3, Cu^2, Sn^2, V^5	(168)
partial for Mn^2 Fe^2 V^4 Ni^2 Ag Hg	
none for Na K Be^2 Mg^2 Ca^2 Zn^2 Co^2 Cr^3 Cd^2 Al^3 As^5, Pb^2	
Carbonyl reagent	
with pyruvate acetaldehyde acetate	(168)
not with glucose, lactic acid	
Complex with SH	
with cysteine glutathione BAL and other simple mercaptans	(168)
Protein binding	
with serum arterial mash egg albumin polypeptides	(436)
not with casein, mixed amino acids	
Enhances	
monamine oxidase (10^{-4})	(Table XIII)
Anti-enzyme	
for histaminase (10^{-8}) stronger than guanidine thiosemicarbazide weaker than hydrazine aminoguanidine equal to semicarbazide	(172)
for DOPA decarboxylase moderate (10^{-4})	(Table XIII)
for succinic dehydrogenase cholinesterase poly phenol oxidase none	(111)
Combination with	
no primary amines or simple amino acids	
lyophilized angiotonin	(168)
Blocking Actions In Vivo	
Weak for epinephrine norepinephrine arterenone tyramine isoamylamine angiotonin	(168)
Strong for pherentasin pervanadyl cadmium barium pitressin	(168 438)
Variable for serotonin according to species (weak in rat and dog strong in cat)	(168 63)
Blocking Actions on Rabbit s Arterial Strip	
Strong for pherentasin angiotonin	(120)
Weak for norepinephrine and other primary amines	(120)

TABLE XV—(*continued*)

Vascular Reactions in Animals	*References*
Dilates constricted vessels renal femoral coronary acts for many hours	(438)
Does not dilate dilated vessels further (as in spinal animal)	(438)
Abolishes constriction caused by Ba pitressin ephedrine ergotamine histamine Privine	(438)
Reactions in Man	
Lowering of plasma cholesterol	(180)
? o lowering of blood pyruvate or total carbonyl	(168)
Apparent loss of Ti in urine	
Mild anemia	(168)
? Histamine release	(172)
Increases cardiac output tachycardia	(425)
Increases renal plasma flow	(426 427)

and side effects due to this ion are variable but resemble in some respects those induced by hydralazine because of the dissimilarity of the chemical structures of the two

TABLE XVI

SOLUBLE COMPLEXES OF THIOCYANATES IN WATER

Soluble	*Partly Soluble*	*Insoluble*
Mn	Pb	Cu
Fe	Hg	Ti ?
Co	Ag	Si
Zn		
Mo		
Ca		
Sr		
Ba		

Hodgman C D ed *Handbook of Chemistry and Physics* 33rd Ed Cleveland Chemical Rubber Publishing Co 1951

TABLE XV

SUMMARY OF PROPERTIES OF HYDRALAZINE OTHER THAN CARDIOVASCULAR

Basic Chemical Reactions In Vitro	*Reference*
Metal binding	
complete for Fe^3 Cu^2 Sn^2, V^5	(168)
partial for Mn^2 Fe^2 V^4, Ni^2 Ag Hg	
none for Na K Be^2, Mg^2 Ca^2 Zn^2, Co^2 Cr^3 Cd^2 Al^3, As^3 Pb^2	
Carbonyl reagent	
with pyruvate acetaldehyde acetate	(168)
not with glucose lactic acid	
Complex with SH	
with cysteine glutathione BAL and other simple mercaptans	(168)
Protein binding	
with serum arterial mash egg albumin polypeptides	(436)
not with casein mixed amino acids	
Enhances	
monamine oxidase (10^{-4})	(Table XIII)
Anti enzyme	
for histaminase (10^{-6}) stronger than guanidine thio semicarbazide weaker than hydrazine amino-guanidine equal to semicarbazide	(172)
for DOPA decarboxylase moderate (10^{-4})	(Table XIII)
for succinic dehydrogenase cholinesterase poly phenol oxidase none	(111)
Combination with	
no primary amines or simple amino acids	
lyophilized angiotonin	(168)
Blocking Actions In Vivo	
Weak for epinephrine norepinephrine arterenone tyramine isoamylamine angiotonin	(168)
Strong for pherentasin pervanadyl cadmium, barium pitressin	(168 438)
Variable for serotonin according to species (weak in rat and dog strong in cat)	(168 63)
Blocking Actions on Rabbit's Arterial Strip	
Strong for pherentasin angiotonin	(120)
Weak for norepinephrine and other primary amines	(120)

Sodium azide which has a strong affinity for metals is a rather transient vasodilator, as is its relative choline azide producing sharp reductions in blood pressure It is said to show differential actions in normotensive and hypertensive rats not depressing blood pressure in the former (175) We have been unable to confirm claims for chronic effects in man

British Anti Lewisite (2 3-dimercaptopropanol BAL) is used clinically to remove trace metals from the body Much is known of its actions (176 177, 178) which do not include affinities for all metals It is a disulfide chelating agent In our hands it has proven effective in causing lowering of blood pressure in American hypertensive patients for periods of a few hours On the other hand British patients have responded with a rise It is prolongedly pressor in normotensive subjects (177) but was depressor in one American hypertensive patient in the hands of others (176) BAL has little clinical use at present in hypertension In cadmium poisoning it will mobilize the metal but binding is weaker than is that of kidney for the metal is deposited and cadmium nephritis results (179) Many other heavy metals are mobilized and removed in the urine

Ethylenediamine tetra acetate is a mild antihypertensive agent in man Given intravenously as the disodium calcium complex it either lowers elevated blood pressure *or* reduces the patient s requirement for ganglionic blocking agents (180) Not a strong chelating agent for many metals it has little clinical use at present Prolonged oral use has led to no toxicity intravenous use has produced signs of zinc deficiency (181) which resembles that of vitamin B_6

Experimental Compounds In anaesthetized rats and other animals a number of compounds having the capacity for binding *or chelating* trace metals lower hypertensive

agents, a common denominator must be present in the actions of both (Table XVII) Little interest in its mode of action has been aroused It can inhibit a number of enzymes, such as zinc-containing carbonic anhydrase and amino acid oxidase (173b) It is antithyroid, all antithyroid agents except those which act by competitive inhibition bind metals

Sodium nitroprusside is an antihypertensive agent of considerable potency when given intravenously, alterations in the course of the disease have been described (174) A zinc reagent, it is a strong metal binder Its chronic toxicity is not known but should become apparent with continued use

TABLE XVII

SIDE EFFECTS OF TWO METAL BINDING ANTIHYPERTENSIVE DRUGS (84 108)

	NaSCN	*Hydralazine*
Action		
in normotension	0	±
in renal hypertension	+	+
enhanced by sympathectomy	+	+
Arthralgia	+	+
Lupus like syndrome		+
Cholesterolysis	+	+
Rhinitis	+	+
Conjunctivitis	+	+
Anti thyroid	+	0
Peripheral neuritis	+	+
Paraesthesias	+	+
Skin lesions	+	+
Headache	+	+
Flushing	0	+

both types of animals D Lowering of blood pressure in both types of rats and depression of the pressor action of norepinephrine E Lowering of the blood pressure of both normotensive and hypertensive rats without greater effect on the latter and without altering the pressor action of norepinephrine (Fig 11)

Mercaptan compounds which showed specific antihypertensive but not sympatholytic effects (Type A) in the rat were

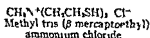


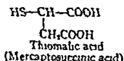


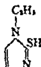




Procaine Salt of β mercapto-propionic acid

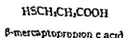


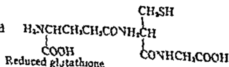




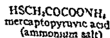
1-ethyl 2 mercaptoimidazole

Compounds showing both antihypertensive and norepinephrine blockade (Type C) were





and to a less extent



blood pressure but do not affect or raise normal blood pressure

Theoretically there are five types of sustained depressor responses of the blood pressure of hypertensive and normotensive rats to the intravenous injection of various active compounds A Little or no effect on normotensive rats with specific lowering of the blood pressure of renal hyper tensive rats, while the pressor action of norepinephrine is unaltered or little affected (Fig 10) B Little effect on blood pressure with depression of the pressor action of norepinephrine in both types of rats C Effect of type A with depression of the pressor action of norepinephrine in

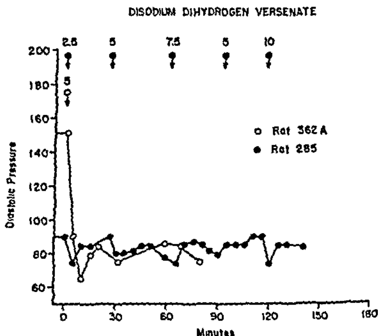
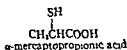
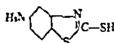


Fig 10 Effect of intravenous EDTA on diastolic blood pressure of anaesthetized rats Note that the normotensive pressure varies little while the renal hypertensive falls with smaller doses Typical type A response

Certain mercaptans were depressor in both types of animals (Type E) although the last two showed significant differential activities


α-mercaptopropionic acid


Monothioglycerol

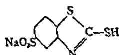

6-amino-α mercaptobenzothiazole

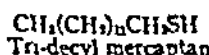

2 3-dimercaptopropanol (BAL) (Fig 12)

Only four mercaptans were sympatholytic (Type B) without depressor effects the last to a lesser degree


Acetonyl mercaptan

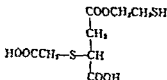

1 thio-2 hydroxy propane

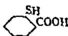

Sodium 2 mercapto-5 benzothiazole sulfonate

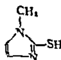

Tri-decyl mercaptan

Five were inactive or pressor the last having a short lived differential action


Cysteine


2 mercaptoethyl hydrogen (carboxymethylmercapto) succinate


Mercaptosalicylic acid


Tapazole


Pantetheine

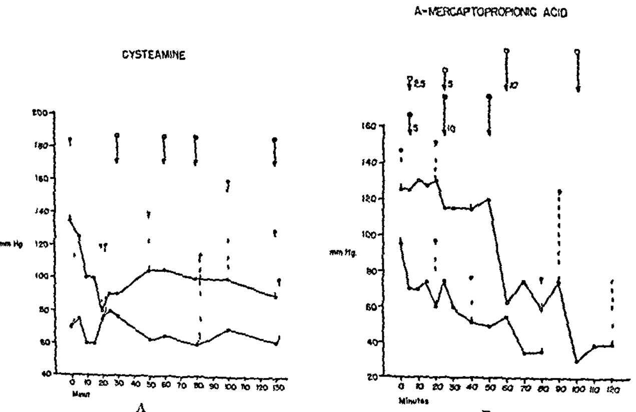
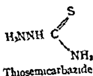
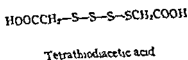
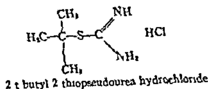


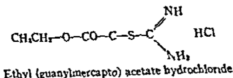
Fig 11 Effect of *intravenous* sulfhydryl compounds on the diastolic pressures of anaesthetized rats A Cysteamine 5 0 mg Only hypertensive pressure is depressed (Type A) B α Mercaptopropionic acid Both types of pressures are depressed (Type E) β-Mercaptopropionic acid gave a type A response The dotted lines denote the rises after 0 3 γ norepinephrine The dose of cysteamine at 0 minutes was 5 0 mg

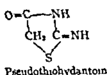
A group of sulfur-containing compounds were similarly divided Type A activity was demonstrated by













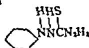


FIG 12 A. Transient effect of 2 3 dimercaptopropanol (BAL) on systolic pressure of renal hypertensive rat. Blood pressure was measured by the foot cuff method using a photoelectric cell The open circles are measurements made 2 3 and 4 hours after the injection the closed circles 24 hours later Note increasing tolerance"

B Effect of BAL on blood pressure of a 59-year-old patient receiving hydralazine (5968) and hexamethonium chloride (C_6) in too low doses to produce normotension Doses are indicated at the top BAL, 50 mg every four hours intramuscularly was given for four doses. At the bottom urinary excretion of hexamethonium ion and hydralazine are shown The solid black areas represent free urinary hydralazine the open areas that bound to sulfhydryl All excreted hydralazine was bound after BAL was given (From Perry H M Jr Schroeder H A. and Morrow J D *Am J M Sc* 228 405 1954)



A

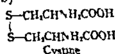
L.H. ♂

C_6 mg/hr
250
125
0
5968 mg/hr
50
25
0
BAL
B P mm Hg
225
200
175
150
125
100
Urine C_6 mg/hr
20
10
0
Urine 5968 mg/hr
3
2
1
0
16 17 18 19 20 21 22 23
N '52

B

Fig 12 *(See facing page for description)*

There was no activity exhibited by oxidized glutathione nor by



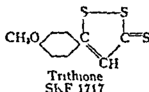


One compound of interest contained three ethyl mercaptans on a quaternary ammonium nitrogen it was made on the possibility that ganglionic blockade as well as mercaptan effect might result However it acutely raised the mean diastolic pressures of normal and hypertensive rats 36 and 15 mm Hg respectively producing the usual differential mercaptan effect of a depression of 6 and 32 mm respectively at the end of 2 hours Another of special interest had the basic structure of hexamethonium ion with an ethyl thiopseudourea group on each quaternary nitrogen Although listed as Type E it depressed the mean diastolic pressure of 5 normal rats 36 mm Hg and that of 5 hypertensive rats 86 mm in doses of 1 0 to 1 5 mg Possibly ganglionic blockade was combined with another action on the renal pressor mechanism

Examination of the structures reveals that antihypertensive or depressor activity was confined to those aliphatic compounds having a terminal sulfhydryl *group or SCNH* in the molecule unencumbered *by a heavy salt* aromatic compounds containing SCN were likewise active Such compounds usually bind metals sulfur nitrogen binding being strongest with Cu Ni Ag Cd and contiguous heavier elements in the periodic table These results suggest that possibly some copper enzyme was altered or inactivated causing the pharmacological activities of the compounds (182)

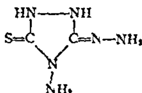
If *this* surmise be true the next step was obviously to test known chelating agents in the same system preferably those not *metabolized* If they were active obviously a metalloenzyme was altered.

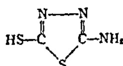
Type C activity was shown by only one

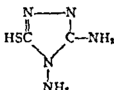


Trithione
SKF 1717

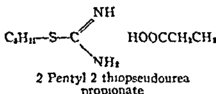
Partial sympatholytic with good antihypertensive activity was exhibited by





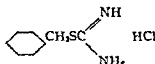


Type E activity occurred after the following the last two showing differential effects

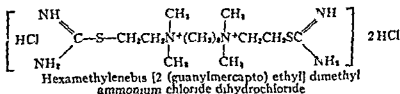


2 Pentyl 2 thiopseudourea propionate





2 Benzyl 2 thiopseudourea hydrochloride



Hexamethylenebis [2 (guanylmercapto) ethyl] dimethyl ammonium chloride dihydrochloride

In order further to control the studies various pyridoxylidene metal amino complexes were subjected to the same test. Selective Type A effects were observed with the copper tyrosine nickel arginine aluminum phenylalanine and possibly the cobalt phenylalanine complexes *No ef*

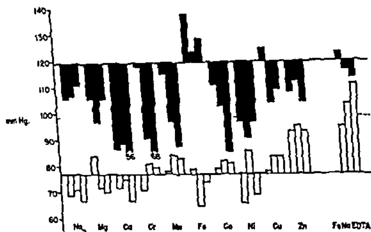
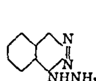
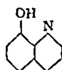


Fig 13 The effects of a series of increasingly tightly bound metal ethylene-diamine *tetra* acetates on the diastolic pressures of groups of hypertensive (upper bars) and normotensive (lower bars) anesthetized rats. The first bar for each metal complex represents the change 20 to 30 minutes after one intravenous injection of 5 mg the second and third changes a like interval after subsequent injections Mean chang s are shown each group representing at least 3 and usually 4 or more rats All complexes were dihydrogen metal except for ferric as shown on the right. Note the comparable differences in hypertensive (mean diastolic pressure 119 *mm.* Hg) and normotensive animals (mean diastolic pressure 77 mm Hg) as well as the small effects with sodium and magnesium complexes which can alter calcium levels in blood, and the decreased effects with nickel copper zinc and trivalent iron Metals are listed in order of atomic number The ferrous was probably partly ferric. (*From* Schroeder H A. and Perry H M Jr *J Lab & Clin Med* 46 416 1955)

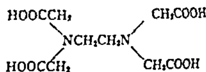
Activities of Type A were shown by the strong chelating compounds



Hydralazine



8 hydroxyquinoline



Ethylenediamine tetraacetic acid (EDTA)

and Perma Kleer a polyaminocarboxylic acid resin with chelating qualities Pressor activity was exhibited by the weaker chelating agent, 1 6 tolylbiguanide Therefore one or more metals must have been removed presumably from metalloenzymes, since EDTA, at least is not metabolized

In order to ascertain which metal or groups of metals might be chelated advantage was taken of the different stability constants of EDTA and various metals of the first transitional series Figure 13 shows the results and Table XVIII the stability constants Any metal removed from tissues must have displaced one with a lower stability constant Aside from the ferrous chelate which readily oxidizes to ferric in solution it is evident that those with higher constants than 16 1 (for cobalt) are not active, these included the nickel zinc and copper chelates Since nickel has no known function it is reasonable to assume that ferric iron zinc or copper was displaced from tissues by the chelating compound

In order to determine whether or not the metal ions themselves showed activity small amounts of the chlorides were injected (Fig 14) Only ferrous cobaltous, cupric and zinc were active Cobalt is a known vasodilator and zinc salts cause flocculation of plasma proteins which may have accounted for the obscured effects Suspicion therefore rests upon copper or zinc as being involved in the maintenance of renal hypertension in the rat

pressure of 4 hypertensive patients monothioglycerol was apparently inactive a trithione (SKF 1717) given orally appeared inert BAL, however exhibited depressor activity in 6 hypertensive patients when given every 4 hours in doses up to 5 0 mg per kg the effects were relatively short lived (2 to 4 hours) We did not observe a pressor effect after this material was given (183)

Comment This common denominator of the antihyper

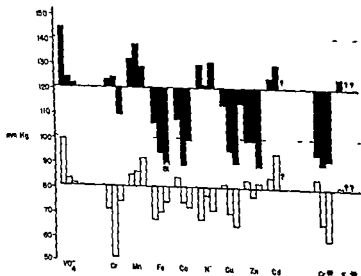


Fig 14 The effects of a series of metal ions on the diastolic pressures of groups of hypertensive (mean diastolic pressure 121 mm. Hg) and normotensive (mean diastolic pressure 81 mm Hg) anesthetized rats similarly treated. Doses were 0 2 mg For obvious reasons sodium and calcium salts were not given The pressor action of pervanadate is indicated as well as are the effects of cadmium and chromic chlorides. Note the differences between the ions and their complexes as shown in Figure 13 especially in regard to chromous manganous ferrous nickelous zinc, and ferric iron which was inert in large doses (From Schroeder H A and Perry H M Jr *J Lab & Clin Med* 46 416 1955)

TABLE XVIII

SIGNIFICANT EFFECTS ON DIASTOLIC BLOOD PRESSURE OF METAL CHELATES (15 MG) AND ION (0 6 MG) IN HYPERTENSIVE RATS

Me^{++}	*Atomic No*	*Log* K_1	$H_2MeEDTA$ *(mm Hg)*	$MeCl_2$ *(mm Hg)*
Na_2	11	1 66	—	—
Mg	12	8 69	—	—
Ca	20	10 59	−63	—
Cr	24	13 00	−51	—
Mn	25	13 47	−31	—
Fe	26	14 22	—	−40
Co	27	16 10	−33	(−21)
Zn	30	16 58	—	−29
Ni	28	18 45	(−22)	—
Cu	29	18 38	—	−28
$Fe^{+++}Na$		25 00	+33	—

Log K_1 is an index of the stability of the chelate the higher values being more stable The figures were taken from Sequestrene a publication of the Alrose Chemical Co Providence quoting Scharzenbach *et al* That for chromous is not exactly known

EDTA—ethylenediamine tetraacetate

fects were observed with three glutamic acid and two phenylethylamine complexes Copper again comes under suspicion

These same effects were observed in renal hypertensive dogs, by the use of BAL and hydralazine (Fig 15) In renal hypertensive dogs BAL (5 mg per kg) injected intramuscularly produced definite but transient (2 to 6 hour) depression of blood pressure as did sodium thioglycolate intravenously When BAL was injected with 1 hydrazinophthalazine a moderately active depressor substance, the effects were enhanced Only four of these compounds were sufficiently studied to give to human beings Cysteine caused no demonstrable alteration in the blood

tensive compounds not acting on nerves that is the ability to bind trace metals is not confined to this class of drugs In fact most modern drugs have this ability Their differences lie in their relative affinities for the metals in question their dispersing powers and their transport to other sites The striking coincidence however of metal binding properties and effect lead to only one conclusion that one or more metals are bound and altered from metallo-enzyme sites and that abnormal trace metals may indeed be acting upon enzymatic mechanisms to produce hypertension A consideration of how they may act will be discussed in following chapters

THE EFFECT OF ANTIHYPERTENSIVE AGENTS ON NEPHROGENIC EFFECTOR SUBSTANCES

A method for screening antihypertensive agents involves the isolated rabbit aortic strip a spirally cut piece of smooth muscle which contracts when pressor substances are applied (120) Substances acting mainly on norepinephrine and other primary amines acting on more complex pressor substances and showing general inhibition of all types can be evaluated While many agents tested cannot be applied to man their activities can be evaluated readily on isolated muscle and in the hypertensive animal A substance which is nontoxic inhibits pherentasin and lowers the blood pressure of hypertensive rats while not affecting normotension is obviously of therapeutic interest

Pherentasin Using the isolated rabbit aorta suspended in oxygenated Ringer s solution a number of these substances have been tested for their activities against pherentasin The relative degrees of inhibition are indicated in Table VII All of the *metal binding* agents are inhibitory On the possibility that pherentasin may be an adrenergic agent a number of sympatholytic substances

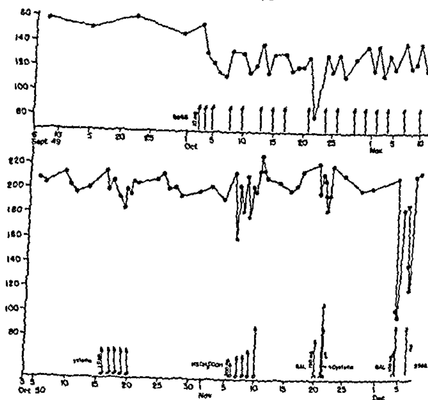


FIG 15 Effect of various substances on the mean blood pressure of a renal hypertensive dog 0 35 The left renal artery was constricted in August 1949 causing moderate hypertension *Upper* 1 Hydrazinophthalazine (5968) injected intravenously Note the effect when given at less frequent intervals than daily blood pressure falling for 24 hours and then rising in 48 Only small doses were required During the subsequent year mean blood pressure rose *Lower* Cysteine intravenously caused only insignificant variations sodium thioglycolate more pronounced but transient ones BAL intramuscularly alone and combined with intravenous cysteine similar depressions but BAL combined with 5968 produced marked responses Both the immediate (2 to 6 hour) and late (24 hour) responses to these substances are shown Only BAL and 5968 together produced significant depression for 24 hours The dog s mean blood pressure consistently remained above 200 mm Hg for 5 months after this study when it was given 1 0 gm thiosemicarbazide by mouth and died of convulsions

is not lowered or raised 2) renal plasma flow increases relatively more than peripheral blood flow 3) blood flow through other areas does not change 4) blood viscosity and volume are not altered and 5) there are no toxic manifestations Hydralazine does not fulfill all of these criteria and also causes late toxicity and some unpleasant side effects However it is probably the closest approximation available at the present time the others either being in

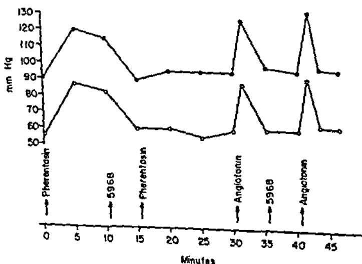


FIG 16 At 0 minutes after a control period of 20 minutes 1 0 unit of pherentasin previously found active was injected intravenously into a rat with the response of systolic and diastolic pressure shown At 10 minutes 1 0 mg of hydralazine (apresoline 5968) was injected intravenously resulting in an immediate fall of blood pressure A subsequent injection of 2 0 units of pherentasin caused little response however 1 0 unit of angiotonin was pressor At 35 minutes 5 0 mg of 5968 was injected the response to another unit of angiotonin 5 minutes later was not inhibited (From Perry H M Jr and Schroeder H A. *Am J M Sc* 228 396 1954)

TABLE XIX

ADRENERGIC BLOCKADE OF VASOACTIVE PEPTIDES (RABBIT AORTIC STRIP)

Blocking Agent	*Pherentasin*	*Hypertensin**	*Serotonin*	*Norepinephrine*
Regitine	0	±		
Dihydroergotamine	0	0	+	+
Iproniazid	sl	sl		
Cocaine	0	0		
Dibenamine	0	0	+	+
Pyribenzamine	0			
Atropine	0			
Amine oxidase	+	+	+	+
Tyrosinase	0	+	0	+

* Probably mainly hypertensin I

were also tested None showed the characteristic alterations of pherentasin activity exhibited by primary amines (Table XIX) Pherentasin was actively inhibited by hydralazine in the intact rat (Fig 16)

Renin and Angiotonin On the isolated smooth muscle system, hydralazine in fairly high doses is antagonistic to angiotonin In the intact animal this antagonism is not demonstrated by doses sufficient to inactivate pherentasin A number of antihypertensive metal binding agents however inactivate angiotonin in the isolated system suggesting that a metal is essential for its activity

Others Hydralazine inactivates sustained pressor principle *as does β mercaptopropionate It also inactivates* pitressin

CLINICAL IMPLICATIONS

A true antihypertensive drug is one which lowers elevated blood pressure to normal without affecting normal blood pressure, in the process of which 1) cardiac output

is not lowered or raised 2) renal plasma flow increases relatively more than peripheral blood flow, 3) blood flow through other areas does not change 4) blood viscosity and volume are not altered and 5) there are no toxic manifestations Hydralazine does not fulfill all of these criteria and also causes late toxicity and some unpleasant side effects However it is probably the closest approximation available at the present time, the others either being in

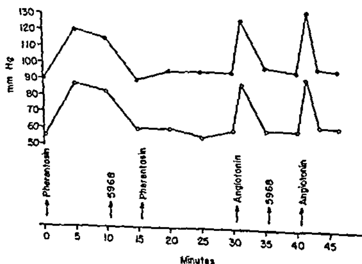


Fig 16 At 0 minutes after a control period of 20 minutes 1 0 unit of pherentasin previously found active was injected intravenously into a rat with the response of systolic and diastolic pressure shown. At 10 minutes 1 0 mg of hydralazine (apresoline 5968) was injected intravenously resulting in an immediate fall of blood pressure A subsequent injection of 2 0 units of pherentasin caused little response however 1 0 unit of angiotonin was pressor At 35 minutes 5 0 mg of 5968 was injected the response to another unit of angiotonin 5 minutes later was not inhibited. (From Perry H M Jr and Schroeder H A *Am J M Sc* 228 396 1954)

experimental stages, or showing excessive toxicities or only transient effects

Toxic effects appearing late are those of the development of a syndrome indistinguishable from disseminated lupus erythematosus, reversible when the agent is omitted (93, 184 187) This syndrome has been characterized by 1) normotension, 2) arthralgia or arthritis, and 3) appearance of elevated cephalin-cholesterol flocculation and thymol turbidity of serum Continuation of the drug causes later manifestations which include anemia, leukopenia, splenomegaly, hepatomegaly, low plasma cholesterol, albuminuria microscopic hematuria, azotemia, pleuritis pulmonary lesions and the appearance of a positive L E test on peripheral blood These dire events appear only when the drug is continued at full doses The many bizarre findings of collagen diseases can occur The syndrome has a seasonal incidence appearing mainly in warm weather Coming on only after 5 months or more of administration of drug it is similar to late toxicity from several other metal binding agents

Since hydralazine is the only known drug which will produce disseminated lupus erythematosus in man and the dog (188) speculation as to its mode of action may help understanding of collagen diseases For some time we were attracted to the hypothesis that this syndrome and perhaps disseminated lupus itself represented a state of depletion from the body of some material important in the health of ground substance Loss of a trace metal or imbalance between essential and abnormal trace metals was considered, in view of hydralazine s known reactions Feeding of several trace metals failed to influence the disease The universal appearance of normotension or hypotension before symptoms appeared was in line with this hypothesis On the other hand, definite hypersensitivity has been observed,

fever acute arthritis and prostration have occurred within a few hours of giving the drug or its analogues (185 189) both to patients recovering from hydralazine disease and to others never receiving the drug before We have seen showers of L-E cells appear in one recovered person who had taken no drug for 2 years and was given small doses In several other individuals reduction of doses has resulted in reversal of symptoms but not of all laboratory abnormalities If the L-E phenomenon is one of hypersensitivity which is not known this explanation for the disease is tenable The question is open but we suspect that its answer will be fruitful of information on hypersensitivity collagen disease and hypertension

We do not know which substance is removed from the body by hydralazine or which enzyme system or group of systems is inactivated The ability to produce this syndrome in man by a drug however suggests that lupus erythematosus itself is an enzymatic disturbance which might be affected by replacement therapy A suggestion of what has been removed in the way of metals may be obtained from trace metal analysis of human urine when hydralazine was given and from cases of known hydralazine disease and known lupus erythematosus (Table XX) The abnormal urines are somewhat low in manganese somewhat high in tin and zinc While these findings may not be pertinent to pathogenesis they deserve study Blood copper levels were not reduced in this syndrome

Of the other known functions of hydralazine carbonyl binding is the most logical to explain the toxicity It is difficult however to conceive of carbonyl binding as leading to depletion There was no diminution in total carbonyl or pyruvate in blood of patients treated with hydralazine Its other function removal of sulfhydryl is also a possible but not probable cause of hydralazine

TABLE XX

URINARY TRACE METAL CONCENTRATIONS* IN DISSEMINATED LUPUS ERYTHEMATOSUS (111)

	Ti	V	Cr	Mn	Ni	Zn	Mo	Ag	Cd	Pb	Sn	Comments
Ch.	<0 05	0 6	5 2	Inter†	1 8	Inter	>50	0 36	1 4	120	38	Lupus
Ev	<0 05	—	—	<5	5 7	180	21	0 29	1 8	15	5 0	Lupus
Sc.	4 0	0 41	0 36	<5	Inter	175	4 4	0 46	3 0	1 1	3 0	Lupus
Mean**	1 37	0 5	2 78	<5	3 75	177 5	25 1	0 37	2 07	45 4	15 3	
Dr	10	4 9	0 50	<5	2 0	175	6 1	0 58	6 0	170	6 8	Lupus
Ha.	3 6	1 3	1 9	<5	1 4	87 5	12	0 33	<0 3	5 3	17	Lupus
Mean	6 8	3 1	1 2	<5	1 7	131 3	9 0	0 46	3 15	87 65	11 9	
St	2 8	1 5	0 34	<5	1 1	159	2 4	2 0	70	0 90	8 8	Hydralazine D sease
Sa.	<0 05	0 11	12	<5	4 4	108	3 9	1 2	0 5	0 50	7 8	Hydralazine Disease
Mean	1 43	0 81	6 17	<5	2 75	133 5	3 1	1 6	35 2	0 70	8 3	
Normal												
Mean	<3 8	<0 63	<0 64	<9 7	<2 8	<67	<14	0 80	<0 86	<5 7	<3 2	
Max.	11	2 2	1 0	37	12	135	29	1 4	4	28	5 6	
Min	<0 05	<0 5	<0 05	<5	<0 05	<5	<0 5	0 23	<0 5	<0 05	<0 5	

*Results are expressed in parts per b llion
**Specimens supplied by Dr A C Corcoran.
†Interference

disease Its known antihistaminase activity and the relation of histamine to hypersensitivity is another possible explanation as is its affinity for pyridoxal

Lest the reader refrain from using this potent agent when it is needed for the prolongation of life we can say that hydralazine disease occurs in less than 10 per cent of patients and then only when relatively large doses are given that it is readily detectable easily reversible and does no permanent harm if the drug is discontinued in time There has been no mortality except in unwatched patients and the drug on the whole is hardly more toxic than many in continuous use for the control of chronic diseases The method of use is discussed in Chapter VIII

Chapter V

FACTORS INFLUENCING THE CONVERSION OF NEUROGENIC TO NEPHROGENIC HYPERTENSION

THE TRANSITION FROM INTERMITTENT TO PERMANENT VASOSPASM

IF THE VASOSPASM resulting from emotional reactions to stress were confined to the reversible phenomena seen in hyperreactors to pain the net effects upon the cardiovascular system would be less important than those resulting from exercise (Unfortunately at some stage *permanent vasospasm*, at first of slight degree then more and more pronounced gradually develops engrafted upon which are the repetitive reversible episodes of neurogenic vasospasm) The key to the understanding of the pathogenesis lies in this change of reversible to irreversible vasospasm therefore we may be allowed to theorize for purposes of orientation The curve of incidence of chronic hypertension in the general population plotted against age (showing a rapid increase in the fifth decade) is consistent with several theories Since all biologic phenomena can eventually be resolved in terms of physics and chemistry we should examine possible basic disturbances in that light The physical explanation—organically induced increase in vascular resistance from sclerosis of all vessels—does not fit the facts although the effects of increased intra arterial tension which both cause cardiac overwork

and which increase the rate of development of atherosclerosis are beginning to be appreciated Of chemical explanations we look for some disturbance of some enzyme system concerned a) in the relaxation of smooth muscle b) the destruction of potential pressor substances, c) the reactivity of blood vessels or d) the metabolism of the kidney

THEORY OF HABITUAL REPETITIVE STIMULI

One idea is that the trigger mechanism for emotional discharge to vasospasm becomes more sensitive as the years pass lesser and lesser stimuli setting off the response In other words an habitual pattern of reaction is set up which becomes more and more active and eventually leads to organic renal vascular disease thereby causing organic renal ischemia This explanation begs the question and is inconsistent with the fact that demonstrable renal vascular disease may be *absent* in sustained hypertension

THEORY OF DEPLETION OF VASCULAR SUBSTANCES

Another theory is concerned with the depletion of substances or the wearing out of the mechanisms which cause reversal of vasospasm i e the relaxation of smooth muscle In other words repetitive stresses accelerate the aging process in smooth muscle making it more reactive There is no evidence for this theory although as Szent Gyorgi has pointed out (190) contraction of muscle involves loss of potential energy relaxation a build up of energy (phosphate) in the contractile elements Therefore slight loss of some substance promoting the restoration of the energy for relaxation may occur with time or possibly inhibition of the mechanisms of energy storage by accumulation of another substance In that event, permanent vasospasm

would result from normally circulating vasoactive substances This idea has no present basis of fact, although the ions calcium, magnesium sodium and potassium are intimately concerned in muscular contraction and relaxation and it is not impossible to believe that imbalances occur with age

The irritability of muscle and presumably smooth muscle depends upon the ratio in extracellular fluids

$$\frac{Na^{+} + K^{+} + OH^{-}}{Ca^{++} + Mg^{++} + H^{+}}$$

When the concentration in the numerator is increased irritability increases when that in the denominator is increased irritability decreases The extracellular concentrations under most conditions can affect intracellular ones Therefore vascular smooth muscle may become more irritable and therefore contract more either with higher sodium and potassium levels, by interference or displacement of calcium or magnesium by another inactive element or by their depletion (192)

INTRARENAL ENZYMATIC MECHANISMS

If all nephrogenic hypertension (except azotemic) is dependent upon the same renal enzymatic alterations it is well to consider and search for reasons as to how this can come about Hypertension has been produced experimentally in animals and resulted in man from a variety of methods of damaging the kidney most of them can be considered as producing ischemia Hypertension has also been produced in dogs with no kidneys kept alive by hemodialysis, whether the mechanism is the same or not is unknown Yet in man with permanent hypertension there

may be no vascular lesions no organic renal disease no organic renal ischemia but functional changes are found which are obviously dependent upon circulating vasoconstrictor substances provoking spasm In the dog spasm is even present distal to renal arterial constriction (10) What is the reason?

To find the answer we must delve deeper into those mechanisms affected by ischemia in order to think of similar ones altered in the functional state Something has happened to the kidneys of patients with sustained functional hypertension which may be similarly affected in organic renal ischemia We look to altered enzymatic mechanisms to supply us with a common denominator Because ischemia is related to oxygen tension and oxygen consumption oxidative mechanisms are the first to be considered Is it possible therefore that some renal oxidative enzyme in man is reduced in function by both organic ischemia and an exogenous accumulating substance? If this were so a population might be exposed uniformly to this substance but only certain members predisposed to hypertension i e those who react to stress by vasospasm might develop permanent disease

Pickering put forth this same idea that a whole population was contaminated but only certain persons developed the disease (Chapter II) This theory is the only one consistent with the known facts and which explains the virtual absence of the disease in many areas of the world For it is likely that a proportion of all human beings react to stress by neurogenic discharges through the sympathetic nervous system

There are two possibilities intimately related which should be explored in order to discover this basic disturbance Both could explain this most important factor One involves vitamin B_6 one trace metals.

Theory of Local Vitamin B_6 Deficiency Vitamin B_6 is a most prevalent coenzyme, causing reactions described before which are essential for life and health Deficiency disease in man has been produced by desoxypyridoxine, a metabolic antagonist which may not compete with all the known functions of the vitamin, the principal lesions were of the skin and included cheilosis chemosis, papular eruptions and dandruff (191) similar to deficiency of other B vitamins or to zinc deficiency (181) Peripheral neuritis has also been produced by isoniazid which causes excretion of this vitamin In monkeys loss of hair, weakness weight loss muscular wasting, microcytic anemia and skin lesions occur Obviously we cannot look to generalized vitamin B_6 deficiency for explaining human hypertension, nor any other disease since most patients lack symptoms and appear in the best of health In young rats however hypertension has been produced by desoxypyridoxine (193 195) Since this coenzyme takes part in all amino acid metabolism, it is possible that a relative or marginal deficiency occurs in the tissues (or kidneys) of populations subject to hypertension being a local deficiency generalized lesions would not occur '

Is there a deficiency of vitamin B_6 in the American diet? Authorities differ in their opinions The need for vitamin B_6 in man has been established but the daily requirement has not, being estimated as more than 1 0 and less than 5 0 mg (191) The vitamin is heat labile (196) and destroyed by light (197) It is destroyed or removed during the processing of foods, which includes canning and cooking (198 199) Simple methods for its estimation, depending upon the growth of certain bacteria or yeasts, are considered as giving values too low (200) or too high (201) compared to rat growth curves *The* answer is difficult to find

The American diet composed as it is of many canned

and processed foods may be marginal with respect to vitamin B_6 during certain seasons of the year, according to the best figures available (201 202) Furthermore all pregnant women appear to be somewhat deficient the growing fetus apparently removing the vitamin from the mother without causing skin lesions (203 205) Army combat rations were found deficient for monkeys and rats (206 207) and a brand of infant food was found deficient causing convulsions (208 209)

As we have said there is obviously no generalized deficiency state which can be recognized in the American adult population Marginal intakes however are possible especially during seasons of the year when the diet is composed largely of processed foods Converting the values in foods described in the literature to include a daily diet we have found that the intake is barely adequate (202) About 0 2 mg per 100 Gm of food is necessary to promote the growth of rats Not many foods contain this much when cooked and it was difficult to calculate a 2 0 mg intake in a sample hospital diet (202)

Why do not pronounced deficiency symptoms appear? Apparently this coenzyme has an affinity for systems where it is most needed for life and less for health Its distribution in organs shows wide variations (201) Perhaps renal deficiency can exist without deficiencies elsewhere, perhaps overloading of one *vitamin* B_6 enzyme system by metabolic products can produce a state of local deficiency without it being manifest in other systems The need for a coenzyme varies as the *load placed* upon the enzyme system as is so well known in the case of vitamin B_1 or thiamin A third possibility is that specific antagonists accumulate with age

Trace Metal Imbalance *The second* theory concerns metalloenzymes There are many in the kidney If deficiency of a metal were produced that enzyme would be

TABLE XXI

URINARY EXCRETION OF TRACE METALS IN NORMOTENSIVE AND HYPERTENSIVE STATES (γ/L) (111)

		Mn	*Zn*	*Mo*	*V*	*Cd*	*Ti*	*Pb*	*Cr*	*Ni*	*Sn*	*Ag*
Normotensive 15 cases	Mean	<9 7	66 8	14 0	<0 6	<1 1	<3 75	3 7	<0 64	<2 78	<3 22	0 80
	Range	<<5-37	<5-135	<0 5-45	<0 3-7 3	<0 5-4	<0 05-10 7	<0 05-28	<0 05-2 0	<0 05-12	<0 5-10 25	0 4-1 4
Untreated hypertensive 16 cases	Mean	54 11	106 3	10 63	1 95	37 0	5 83	14 23	0 88	5 53	8 93	1 21
	Range	<5-305	31-355	2 1-26	<0 2-14 5	<0 5-3 0	<0 5-31	0 93-50	<0 05-4 4	<0 1-16 8	0 5-41	0 3-4 6
Untreated hypertensive 8 cases	Mean	62 6	109 7	7 59	2 86	41 7	3 21	10 3	0 67	3 99	3 47	1 44
	Range	<5-305	5-205	3 8-15 5	0 4-14 5	<0 5-370	1 8-3 3	0 06-50	<0 05-3 1	1 1-6 75	0 5-10 5	0 3-4 6
Same treated	Mean	12 14	89 73	4 54	0 93	3 14	3 97	2 19	0 71	5 78	2 64	0 73
	Range	<5-36	31 170	0 5-10 25	0 27-1 85	<0 5-18	0 8-11	<<0 5-4 9	<0 05-2 6	0 3-28 5	0 3-9	0 24-1 7
Hydralazine disease 2 cases	Range	<5	108-159	2 4-3 9	0 11-1 3	0 5-70	<0 05-2 8	0 5-0 9	0 34-12	1 1-4 4	7 6-8 8	1 2-2 0
Disseminated lupus 5 cases	Mean	<5	154	18 7	1 80	2 5	3 54	123 5	1 99	2 7	13 96	0 40
	Range	<5	87 5 180	4 4->50	0 5-4 9	0 3-6 0	<0 05-10	1 1-170	0 4-2 8	1 4-5 7	3 0-38	0 29-0 58

By ganglion c bl ckade and Hydralazine
From data of Perry and Schroeder

come inactive until the metal were replaced There is no evidence at the present time that specific metal deficiencies exist in man with the possible exception of zinc in under nutrition On the contrary there are many trace metals in American tissues which perhaps are not only unneces sary but undesirable The kidney is notable in this respect (Chapter VI)

(Because an undesirable metal can replace an essential one in an enzyme system and inactivate it *in vitro*, it is probable that such a consequence can occur *in vivo*) In order to determine where to look we must examine the essential and the presumably abnormal trace metals in American human adult tissues and urine (Table XXI) compare them with metals found in infants to discover which accumulate with age and also compare the tissue content of people from areas not exposed to hypertension This subject will be discussed in Chapter VI but examples can be considered here

Cadmium was found in large quantities in adult Ameri can kidneys but not in infancy This metal is nephrotoxic An examination of the inhibitory effects of a number of trace metals upon DOPA decarboxylase and monamine oxidase revealed the following Some inhibition was ex hibited by all in high concentrations but at low (0 1 millimolar) only cadmium and mercury significantly in hibited enzymatic activity of DOPA decarboxylase both inhibited monamine oxidase to less extent (Table XXIII) Both are nephrotoxic and will displace zinc (p 146)

(Any disease which is a function of aging may be influ enced by the gradual accumulation in tissues of those trace metals which appear to show organ selectivity and poor excretion) Any diseases appearing frequently as a function of Western Civilization which are virtually ab sent in uncivilized man may be influenced by accumula

TABLE XXII

CHANGE IN SEVERAL METALS WITH AGE (P P M ASH)*

Decade	No Cases	Kidney						Lung		Liver					
		Ni	*Zn*	*Cd*	*ΔZn-Cd*	*Pb*	*Sn*	*Al*	*Ti*	*Ti*	*Ni*	*Cd*	*Sn*	*Cr*	*Pb*
0	5	6†	1850	0	1850	13	15‡	77	0	0	0†	0	3‡	22	34
1	2	0	2050	160	1890	36	134	60	0	0	0	0	103	15	45
2	1	10	4700	1450	3250	68	33	440	82	14	0	190	66	21	115
3	4	14 5	5800	2750	3070	130	17	>1540	220	7	12	174	38	16	171
4	3	10	5100	2500	2600	115	37	>2400	>740	19	<33	167	124	26	140
5	5	13	6300	3500	2800	96	91	>>2320	>660	22	0	186	33	14	77
6	6	16	7450	4200	3200	96	24	>>2660	>910	10	<4	282	27	35	115
7+	4	51	6050	2850	3200	71	44	>>3000	>900	10	37	385	36	17	77

* After Tipton (231)

† Present in only 1 stillborn

‡ Absent to trace in stillborn Present in 7 week old child only

NOTE These values are indicative of concentration in wet weight of organ ± 10% The remarkable constancy of the differences between zinc and cadmium suggests that as *cadmium is accumulated displacing zinc from enzymes, more zinc enzymes are formed If they were not* this amount of cadmium would cause overt renal toxicity

tion of those abnormal trace metals to which civilized man is exposed Cardiovascular diseases associated with aging therefore may be influenced by accumulation of trace metals in kidney liver blood vessels adrenal or brain

In Table XXII are shown evidences of accumulation of various trace metals or lack of it in American kidneys with age Although the numbers in each decade are small the trends are definite for nickel titanium and cadmium, not so for tin Zinc is accumulated in proportion to cadmium One of these metals could be the culprit although we suspect cadmium because of its prevalence (see p 146)

Other oxidative metalloenzymes in kidney (or elsewhere) might be affected by abnormal exogenous trace metals Two pertaining to the problem of hypertension are listed in Table XXIII (Any enzyme containing free sulfhydryl groups can be inactivated by metal binding thereon thus metalloenzymes are not essential for inactivation by metals) Direct evidence for their participation is lacking but they are shown to call attention to their role in nitrogen metabolism direct or indirect and to their metalloenzyme natures Vanadium and cadmium have striking actions

Theory of Electrolyte Imbalance Small elevations in the serum sodium of hypertensive patients have been reported from time to time (210 211) Their significance is unclear The hypertensive kidney is a salt losing kidney no functional or morphological alterations in adrenal cortex have been demonstrated in the usual case There is evidence however, that the sodium content of arterial walls may be increased causing enough swelling to increase peripheral resistance (212) There is also evidence that the sodium in the body affects vascular irritability in that the peripheral vessels become less sensitive in sodium depletion and more sensitive in sodium repletion and the administration of desoxycorticosterone ace

TABLE XXIII

EFFECT OF METAL IONS ON TWO ENZYME SYSTEMS (GUINEA PIG) (% ACTIVITY) (311)

Metal	Bound by Hydralazine	DOPA Decarboxylase Millimolarity of Metal ion				Monamine Oxidase (Substrate tryptamine) Millimolarity of Metal ion			
		10	1	0 1	0 01	10	1	0 1	0 01
Mg^{++}		103	—	—	—	94	99	99	96
Ti^{+++}		24	82	94	98	87	101	106	103
Ti^{++++}		52	92	103	98	100	100	101	99
V^{+++}	+	11	99	113	97	185	137	118	110
V^{++++}		7	100	99	94	256	154	104	108
V^{+++++}	+	96	—	—	—	127	106	101	99
Cr^{++}*		34	100	—	—	90	102	102	104
Cr^{+++}	0	26	77	86	92	110	111	96	106
Mn^{++}*	+	84	94	—	—	98	91	98	94
Fe^{++}	+	70	100	—	—	106	103	101	102
Fe^{+++}	+	32	106	105	—	106	104	98	103
Co^{++}*	0	69	91	94	94	144	136	109	107
Ni^{++}*	+	33	100	—	—	110	107	102	105
Cu^{+}		60	93	—	—	58	114	101	96
Cu^{++}	+	8	13	91	93	18	110	86	101
Zn^{++}	0	41	83	93	93	93	—	—	—
Cd^{++}	0	2	9	54	89	86	82	88	94
Hg^{++}	+	—	6	58	86 (89)	1	64	102	106
UO_2^{--}		93	105	59	101	127	98	100	97
$Me^{++}Na_2EDTA$									
$Mg^{++}Na_2EDTA$		104	110	—	—	127	116	102	92
Ca^{++} "		103	101	—	—	108	102	106	91
Mn^{++} "		106	107	—	—	126	109	107	103
Fe^{++} "		100	105	—	—	—	117	107	—
Na_2 "		95	94	—	—	125	1 8	115	101
Na_2 " (24 hr)		102	—	—	—	—	—	—	—

Italicized figures represent 20% change Note the specific effects of Cd Hg V Co (cf Table XIII p 88)
Those in parenthesis show dilutions by 10
* Depressor in hypertensive rats as EDTA complex (Figs 13 and 14 pp 105 107)

tate What change affecting sodium intake loss or shift occurs in hypertension is not known There is *no* correlation with salt intake in man although moderately hypertensive rats choose to eat more The alteration must be an esoteric one and may involve potassium and magnesium or possibly calcium as well

Theory of Mechanical Renal Arterial Obstruction In view of the above discussion it is highly possible that enzymatic alterations secondary to organic ischemia and those caused by one or more of the aforementionel factors may be similar If so partial obstruction of a renal artery by atherosclerotic plaques could provide the necessary mechanism for permanent hypertension just as well as could intrarenal enzymatic changes from trace metals or coenzyme deficiency Such obstructive lesions exist and may be more common than realized (145) One can imagine a hypothetical case a man with the ability to react to stress by vasospasm passes through his first five decades only with tachycardia or transient hypertension under the stimulus of an examination In his fifth decade in our civilization he begins to develop overt atherosclerosis plaques of which are deposited by chance or by dynamic design at the mouths of his renal arteries He then develops hypertension caused by some organic renal ischemia and some neurogenic vasospasm As the hypertension increases the rate of development of atherosclerosis these plaques may become larger leading to further renal ischemia and hypertension but without much intrarenal arterial sclerosis He *dies in* his sixth or seventh decade usually of an atherosclerotic complication This sequence of events may be very *common* and does not necessitate trace metal imbalance or other enzymatic disturbance *unless a common* disturbance influences both hypertension and atherosclerosis (Chapter VII) Further

TABLE XXIII

EFFECT OF METAL IONS ON TWO ENZYME SYSTEMS (GUINEA PIG) (% ACTIVITY) (311)

Metal	*Bound by Hydralazine*	*DOPA Decarboxylase Millimolarity of Metal ion* 10	1	0 1	0 01	*Monamine Oxidase (Substrate tryptamine) Millimolarity of Metal ion* 10	1	0 1	0 01
Mg^{++}		103	—	—	—	94	99	99	96
Ti^{+++}		*24*	*82*	94	98	87	101	106	103
Ti^{++++}		*52*	92	103	98	100	100	101	99
V^{+++}	+	*11*	99	113	97	*185*	*137*	118	110
V^{++++}		7	100	99	94	*256*	*154*	104	108
V^{+++++}	+	96	—	—	—	*127*	106	101	99
Cr^{++}*		*34*	100	—	—	90	102	102	104
Cr^{+++}	0	*26*	*77*	86	92	110	111	96	106
Mn^{++}*	+	84	94	—	—	98	91	98	94
Fe^{++}	+	*70*	100	—	—	106	103	101	102
Fe^{+++}	+	*32*	106	105	—	106	104	98	103
Co^{++}*	0	*69*	91	94	94	*144*	*136*	109	107
Ni^{++}*	+	*33*	100	—	—	110	107	102	105
Cu^{+}		*60*	93	—	—	58	114	101	96
Cu^{++}	+	*8*	*13*	91	93	*18*	110	*86*	*101*
Zn^{++}	0	*41*	83	93	93	93	—	—	—
Cd^{++}	0	*2*	*9*	*54*	89	86	82	88	94
Hg^{++}	+	—	*6*	*58*	86 (89)	*1*	*64*	102	106
UO_2^{--}		93	105	99	101	*127*	98	100	97
$Me^{++}Na_2EDTA$									
$Mg^{++}Na_2EDTA$		104	110	—	—	*127*	*116*	102	92
Ca^{++} "		103	101	—	—	108	102	106	91
Mn^{++} "		106	107	—	—	*126*	109	107	103
Fe^{++} "		100	105	—	—	—	117	107	—
Na_2 "		95	94	—	—	*125*	*118*	115	101
Na_2 " (24 hr)		102	—	—	—	—	—	—	—

Italicized figures represent 20% change Note the specific effects of Cd Hg V Co (*cf* Table XIII p 88)
Those in parenthesis show dilutions by 10
* Depressor in hypertensive rats as EDTA complex (Figs 13 and 14 pp 105 107)

guish between these conditions and functional neurogenic vasospasm they can be presumed to react in the same way Therefore in late stages arteriolar nephrosclerosis caused by the hypertension produces organic renal ischemia which sustains the hypertension As discussed previously we probably cannot use this mechanism to account for middle stages of sustained hypertension because organic lesions are often not present.

Similarly the tubular part of the nephron may be un able to distinguish the difference between organic arterial and arteriolar narrowing from these causes or from intra renal arterial obstruction by scars (pyelonephritis) and glomerular obstruction (nephritis and glomerulosclerosis) The locus of the mechanism reacting to renal ischemia may be postglomerular (tubular) or it may be in the juxta glomerular apparatus which lies around the afferent arteriole In the latter case chronic glomerulonephritis might not be expected to cause hypertension until fairly widespread renal degeneration had occurred This may be the usual situation

Comment One can only guess at which factor operates in a given hypertensive patient. There may be several others not mentioned The theory of vicious cycles or cybernetics is quite prominent in much of what has been said as it is in many pathologic states and normal *met abolic* pathways (which are far from vicious until dis *turbed*) This mechanism which transforms intermittent neurogenic vasospasm into permanent nephrogenic and neurogenic hypertension, is the killer' Therefore it becomes of foremost importance to understand it for treatment If we could *counteract this* one mechanism and break the cycle perhaps hypertension would be a mild relatively nonfatal *but interesting* physiologic abnor mality

more, cholesterol emboli in the kidneys have occurred from plaques (213), associated with hypertension

The renal hemodynamic picture of hypertension in older persons is that of the major resistance being on the arterial side of the glomerulus (214) contrary to that seen in younger people where it is predominantly in the efferent arterioles If the disease begins in the 50 s and 60 s such sequential events are likely pathogenetic features, although it is now impossible more than to guess which comes first A vicious cycle of this sort involves the initiation of nephrogenic hypertension by local atherosclerosis and the progression of atherosclerosis by hypertension with the predisposing factor (neurogenic vasospasm) present, however, for the previous lifetime of the individual

Sustained chronic hypertension causes arteriolar nephrosclerosis characterized in order of appearance by 1) thickening of the glomerular capsule 2) thickening of the glomerular intercapillary substance 3) thickening and hypertrophy of the walls of the arterioles 4) intimal thickening and 5) fibrosis and hyalin degeneration of the walls of arterioles and small arteries In neurogenic hypertensive dogs these alterations take 2 to 4 years to develop (7) in man with chronic hypertension little or no changes are apparent in half of biopsies (159) while almost all show it at necropsy (2) When pheochromocytomata act as the neurogenic factor for long enough, arteriolar nephrosclerosis is the frequent result and nephrogenic hypertension may remain after removal of the tumor

When arteries and arterioles are narrowed by permanent sclerotic changes blood flow to nephrons is obviously reduced at normal blood pressure levels It matters little to a nephron whether its flow is cut down by a single aortic and renal arterial plaque or by organic narrowing of its afferent blood vessel Perhaps its tubules cannot distin

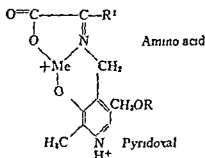
TABLE XXIV

VARIOUS EFFECTS OF PYRIDOXINE IN MAN WITH ESPECIAL REFERENCE TO ANTAGONISM TO METAL-BINDING AGENTS

Clinical Finding	*Induced by Metal binding Drug*	*Relieved by Vitamin B_6*	*No Reports*	*Remarks*
Convulsions	Isoniazid	Yes	1	
Convulsions	Semicarbazide	Yes	1	
Peripheral Neuritis	Isoniazid	Partly	1	Local disorder
Leukopenia and agranulocytosis	Thiouracil	Yes	5	
	Sulfonamides	Yes	2	
Peripheral Neuritis	Arsenic	Yes	1	
Cheilosis Chemosis etc	EDTA	No	1	Zinc deficiency
Same	Desoxypyridoxine	Yes	2	
Same	Spontaneous	Yes	8	
Seborrheic Dermatitis	Desoxypyridoxine	Yes	2	
Same	Spontaneous	Yes	4	Applied locally

Vitamin B_6 Selected Annotated Bibliography 1954 Merck & Co

Can we bring some of these ideas together into one theory? In the case of enzymatic mechanisms, we can For pyridoxal phosphate is believed to contain a metal necessary for activity chelated to the pyridoxal amino acid complex in the following manner (215)



We have demonstrated that cadmium and mercury can selectively inhibit at least one pyridoxal enzyme, probably by competing with the essential metal just as a number of strong metal binding agents also inhibit this same enzyme (Chapter IV) probably by removing the essential metal Therefore, certain trace metals can be biochemically interrelated with certain pyridoxal enzymes It is possible that not only the decarboxylases which produce the vasoactive and cerebroactive primary amines serotonin tryptamine tyramine, isoamylamine dihydroxyphenylethylamine from amino acids but also transaminases may be inhibited by abnormal trace metals (Table XXIV)

CLINICAL IMPLICATIONS

The existence of organic narrowing of renal arteries and arterioles must be in mind during every attempt to reverse or control hypertension whether by drugs or surgery No available therapeutic measure known will dilate renal arteries and arterioles more than they can dilate through smooth muscular relaxation scar tissue will not

mechanisms are unknown but the clinical and laboratory evidence that adrenal steroids cause hypertension in certain cases is clear The reader should remember, however, that there is no evidence that the adrenal cortex is over active in most cases of neurogenic or nephrogenic hypertension but that isolated instances in which it plays a definite and perhaps primary role are known

Experimental Steroid Hypertension For many years it has been recognized that desoxycorticosterone (DOCA) a salt retaining hormone will cause hypertension in rats when added salt is given (216 217) Likewise a syndrome similar to toxemia of pregnancy can also be produced relieved or prevented by hydralazine (218) Feeding of salt alone in excessive quantities can produce rat hypertension (219) vascular lesions result The amount of steroid and the amount of salt necessary to produce this disorder are far beyond physiologic limits DOCA is pressor in renal hypertensive dogs (220) and hypertensive patients (221) Salt restriction apparently induces adrenal cortical hyper activity (222)

Effect of Experimental Nephrogenic Hypertension on Adrenals Adrenal hypertrophy accompanies experimental nephrogenic hypertension (223) Furthermore rats with moderate hypertension voluntarily drink more saline than do normals or their paired severely hypertensive mates (224) This increased requirement for salt may be a reflection of the salt losing tendencies of ischemic kidneys already discussed in Chapter IV

Relation of Adrenal Cortex to Medulla It may not be a coincidence that the adrenal medulla concerned with the release of epinephrine and the cortex concerned with sugar salt and sex, are enclosed in the same gland. There is an intimate relationship between the two hormones acting on vascular smooth muscle There may be a further

become more elastic This statement may not hold true however, for cholesterol filled atheromata, which probably can be partly absorbed under the proper conditions

Fortunately, the cases of severe hypertension which become azotemic when the blood pressure is lowered are rare When present azotemia may be worsened The existence of renal arterial constriction can make therapy difficult, however, intrarenal constriction beyond the obstruction probably can be quite readily opposed

The existence of organic narrowing of other major arteries to myocardium and brain must be in mind during every attempt to reverse or control hypertension, for a lowered peripheral pressure may cause ischemia beyond the obstruction These circumstances are fortunately uncommon

Because hydralazine and similar compounds appear to attack the factor converting intermittent into permanent vasospasm in time, this drug is indicated in all patients with sustained hypertension who are able to tolerate it without symptoms of sensitivity Whether or not it helps to restore a disturbed enzyme system to normal function, or merely makes abnormality more abnormal, is not known at this time Its reactions on DOPA decarboxylase suggest that amino acid decarboxylation would be suppressed by the kidney thus preventing the formation of amines its actions on monamine oxidase suggest that it can promote the destruction of amines its inhibition of pherentasin suggests that it specifically inactivates the one pressor substance found in the hypertensive state

ADRENOCORTICAL MECHANISMS

One possible factor which may influence the conversion of intermittent to sustained vasospasm lies in the adrenal cortex and in its influence on electrolyte balance The

readily when deprived of sodium conductance is restored by a number of quaternary ammonium compounds (229) Whether or not increased conductance occurs when there is an excess of intraneuronal sodium is not known The decreased sensitivity and the sometimes lowered blood pressure seen when dietary salt is severely restricted may perhaps be explained on this basis

These interactions between nerve transmission salt vasoconstrictor substances and steroids can explain some of the clinical findings which appear on the surface to be inexplicable Normal vasomotor tone normal discharges of sympathetic fibres normal amounts of norepinephrine can produce generalized vasospasm when the vascular smooth muscle becomes hypersensitive through salt and steroids Removal of salt or steroids may restore sensitivity to normal Excessive vasomotor tone excessive discharges of sympathetic fibres excessive amounts of norepinephrine formed at nerve endings can produce a much greater degree of generalized vasospasm when the vascular smooth muscle becomes hypersensitive Removal of salt or steroids restores sensitivity to normal but does no more than partly reduce the vasospasm to a lesser level to achieve strict normality requires additional restoration of sympathetic activity to normal When the vasospasm is in part caused by circulating humoral pressor substances restoration to a normal state is impossible unless these substances are inactivated

Clinical Findings Many but not all patients with adrenal cortical adenomata or hyperplasia have hypertension Other steroid producing tumors may also be associated with hypertension Hypertension is uncommon but not unknown in virilizing tumors we have seen it regress on surgical removal of the tumor Cushing s syndrome is

more basic, relationship between salt retaining hormone and the transmission of nerve impulses along sympathetic fibres affected by sodium or potassium

1 The administration of DOCA increases the sensitivity of vascular smooth muscle to epinephrine and norepinephrine (38)

2 The vessels of the patient with Addison s disease show a relatively low reactivity to injected epinephrine and norepinephrine (225, 226)

3 Salt restriction decreases these sensitivities, salt repletion increases them

4 The hypertensive, but not the normotensive, individual responds to intravenous DOCA by a rise in blood pressure (221)

There are two possibilities to explain these findings The first is concerned with the smooth muscle fibre the second with unmyelinated sympathetic nerve fibres Certain adrenal cortical steroids apparently act at the cellular level regulating the amount of sodium, potassium and possibly magnesium within the cell (227 228) At least there are rather profound alterations in cellular content of cations when salt retaining hormones are given or are formed in excess in extracellular fluid there is apt to be hypernatremia hypokalemia and alkalosis of variable degrees If smooth muscle cells were so affected, the result might be hyperirritability of the fibres with excessive responses to vasoconstricting impulses either neurogenic or mediated through circulating pressor substances Perhaps the intracellular edema found in the arteries of some hypertensive individuals (212) is on this basis

Another theory involves the effect of sodium or potassium on nerve transmission through unmyelinated fibres The fibres of primitive animals do not transmit impulses

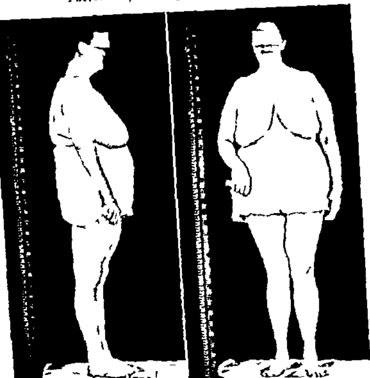


FIG 17 Central obesity menstrual irregularities low sweat salt (<20 mEq/L) and hypertension Rapid weight gain mild hirsutism easy bruisability and moderate diabetes were also present This complex has been named the endocrine hypertensive syndrome for want of a more definitive word The blood pressure was highly responsive to salt restriction (3 3c)

They may be recognized by the presence of central obesity and hypertension in women menstrual irregularities are common (Fig 17) The condition has been seen in families (3) Presumably their hypertension is influenced by an overproduction of aldosterone or other salt retaining hormones which decreases the sodium in sweat to low levels We must hypothecate the chain of events in the

not invariably associated with hypertension primary aldosteronism, in which there is overproduction of salt retaining hormone, appears to be

The administration of cortisone and adrenocorticotropic hormone (ACTH) is sometimes followed by hypertension In such cases, we must assume that sensitivity of vascular smooth muscle increased because of the mild salt retaining side effects of these agents which are not of themselves primarily concerned with salt In the presence of a normal or decreased sympathetic tone cortisone should be inactive in this respect Hormones with lesser salt retaining qualities hydrocortisone, metacortandren etc are less active DOCA, on the other hand, produces hypertension in a fair number of Addisonian patients treated with large amounts of salt

Therefore, the pressure raising activities of steroids and salt, according to this theory are not primary qualities residing in the substances themselves but depend principally upon the state of the neurogenic control of vasoactivity If sympathetic tone is elevated, they elevate pressure If sympathetic tone is low, they do not *unless* excessive hyperphysiologic amounts are given Abnormal amounts of any hormone can cause profound derangements which would not occur with physiological replacement

There is a group of patients now being better described which appears clinically to show excessive adrenocortical activity of two or three types of the hormones concerned with salt, sugar and sex There are many clinical variations from the normal, extending from minor degrees to the borderline of full blown Cushing s syndrome So far, all have had either adrenal cortical adenomata often small, or pituitary basophilism with adrenal hyperplasia

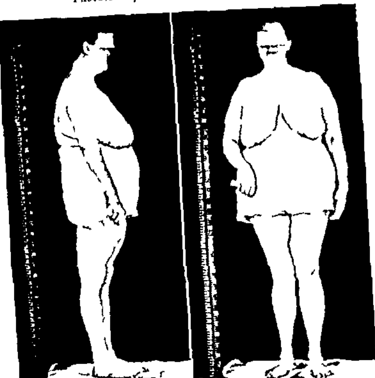


Fig 17 Central obesity menstrual irregularities low sweat salt (<20 mEq /L) and hypertension Rapid weight gain mild hirsutism easy bruisability and moderate diabetes were also present This complex has been named the endocrine hypertensive syndrome for want of a more definitive word The blood pressure was highly responsive to salt restriction (3 3c)

They may be recognized by the presence of central obesity and hypertension in women menstrual irregularities are common (Fig 17) The condition has been seen in families (3) Presumably their hypertension is influenced by an overproduction of aldosterone or other salt retaining hormones which decreases the sodium in sweat to low levels We must hypothecate the chain of events in the

absence of exact hormonal measurements A small adenoma forming only aldosterone will produce hypertension hypokalemia and alkalosis One forming both aldosterone and hydrocortisone or cortisone like hormones will produce hypertension and central obesity with the resultant muscular weakness thin skin and possibly menstrual abnormalities One forming both hormones plus androgens will induce in addition hirsutism an enlarged clitoris menstrual irregularities muscular hypertrophy maleness and the like There can therefore be seven clinical types only four of which are associated with aldosterone and hypertension four with central obesity and four with androgenic overproduction While the distinctions between cases are not as simple as outlined here since a single hormone may have the minor side effects of another this idea is worth considering from clinical grounds and can explain the presence or absence of these different clinical manifestations In aldosteronism the tumor is probably derived from cells of the zona glomerulosa The severity of the manifestations naturally depends upon the amounts of hormones produced in excess

Hypertension and Hyperaldosteronism Recent studies have shown that there is a mild hyperaldosteronism in severe and malignant hypertensive patients as evidenced by the salt retaining properties of urinary extracts in adrenalectomized rats (230) If this is so it is possible that vascular hyperreactivity is dependant upon a slight excess of this hormone However taking into consideration all of the data concerning the adrenal cortex in hypertension this theory is hardly tenable

The hypertensive kidney loses salt under a load and the more severe the hypertension in terms of nephrosclerosis the greater is the tendency to lose salt Since salt de

pletion or some electrolyte abnormality may be the stimulus to the formation of aldosterone it is possible that the mild aldosteronism measured may be merely a reflection of this salt losing tendency which probably has its seat in the kidney Slightly excessive production of aldosterone could be predicted from knowledge of chronic renal salt loss Obviously this form of adrenal cortical hyperfunction would cause if a primary initiating factor salt *retention* by the kidney a phenomenon opposite to what is actually encountered

CLINICAL IMPLICATIONS

Obviously patients with hypertension influenced by adrenal cortical overactivity should respond to restriction of salt by a lowered blood pressure They do The fact that severe salt restriction will not influence severe hypertension secondary to organic renal disease argues against the role of the adrenal in this state Severe salt restriction can occasionally influence neurogenic hypertension probably because vascular reactivity to sympathetic discharges is decreased But these results do not mean that all human hypertension is dependant upon steroids and salt on the contrary these cases are in a minority The reader must remember the simple fact that dietary salt restriction of severe degree causes overactivity of the adrenal cortex and that the usual hypertensive kidney is a salt losing kidney to an extent dependant upon the degree of renal damage or renal ischemia

The clinician does well to recognize cases of aldosterone hypertension for treatment of them may differ radically from that of the usual case of neurogenic or nephrogenic hypertension Although eventually arteriolar nephrosclerosis develops a lesion dependant only on diastolic hyper

tension from any cause, it seems slow to occur in these cases and is apt to be less severe than in other types (3, 4) Salt restriction antiadrenal hormones and adrenalectomy are logical methods to use if diagnosis can be accurate None of these measures is necessary nor justifiable in other types Therefore their recognition becomes of practical significance The measurements of sodium in sweat (3) or saliva and of specific steroids in urine or blood are specialized diagnostic procedures for such cases

Chapter VI

TRACE METALS AND CARDIOVASCULAR DISEASE

INTRODUCTION

BECAUSE of the strong suggestion that trace metal im balances may be involved in some of the chronic diseases to which the people of Western Civilization are exposed a chapter on this subject is in order To be con sidered are the relations of metalloenzymes to the problem the concentrations of essential trace metals in human tissues the presence and amount of abnormal metals and from whence they may come and the possibility of their interference with metalloenzymes to such an extent that they cause chronic diseases Because this subject repre sents a new frontier in Medicine vast gaps in knowledge exist but the pattern is clearing

By trace metals we will consider only those present in small or relatively minute amounts and not discuss the bulk metals sodium potassium magnesium and cal cium nor iron which has an intermediary position between ubiquitous elements and trace metals All bulk metals probably take part in enzymatic reactions or in exchange mechanisms trace metals are often confined to more specialized systems If interference with one of the bulk metals occurred in the body profound toxicity would re sult For example should all magnesium enzymes be in hibited intermediary metabolism would cease if calcium were displaced muscular relaxation would cease Partial

inhibition of some of these systems undoubtedly occurs in disease, but many can be recognized

There are many metalloenzymes in mammalian tissues but only five essential trace metals have been identified manganese cobalt, copper, zinc and molybdenum Deficiency of one of these trace metals in a metalloenzyme either by depletion or displacement by another more or less active metal can be expected to lead to a profound metabolic disturbance induced in a very basic and discrete level There is growing evidence that arterial hypertension and possibly atherosclerosis may be influenced by trace metal imbalances induced by exposure to and accumulation of abnormal trace metals resulting from products confined to Western Civilization

METALS CONCERNED IN METALLOENZYMES

For a metal to be reactive in oxidation reduction mechanisms it must contain at least two valence states which are fairly readily transposed The essential metals iron manganese cobalt and copper fit the requirement as do titanium vanadium, chromium and nickel of the first transitional group For a metal to chelate* readily a requirement for enzymatic activity (232), it should usually

* The definition of chelation and complex formation can best be given by quoting from Martell and Calvin (233) When a metal ion combines with an electron donor the resulting substance is said to be a complex or coordination compound If the substance which combines with the metal contains two or more donor groups so that one or more rings are formed the resulting structure is said to be a chelate compound or metal chelate and the donor is said to be a chelating agent The electron pair bonds formed between the electron accepting metal and the electron-donating complexing or chelating agent may be essentially ionic or essentially covalent depending on the metals and donor atoms involved Without further considering the nature of bonds simple examples of complex formation and chelation are represented schematically as follows

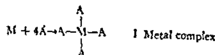
have a coordination number of 4 or 6 an index of the number of donors of the chelate with which the metal will combine Thus magnesium aluminum vanadium chromic ion manganous and manganic ferrous and ferric, cobaltous and cobaltic nickelous and nickelic tin and lead have coordination numbers of 6 while zinc cupric cadmium mercury silver gold have one of 4 and molybdenum of 8 Those of titanium and scandium have not been determined (233) Some functional groupings which bind metals are carboxyl hydroxyl carbonyl amino (primary secondary tertiary cyclic tertiary) sulfhydryl thioether sulfonate and phosphonate (232)

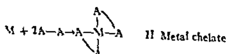
Principles of Chelation The general rules regulating the stability of metal chelates according to Bailar (234) are as follows

1 Ring structures involving metals and organic configurations have increased stability

2 Five membered rings in the absence of double bonding and six membered rings in its presence are the most stable

3 Fused rings that is configurations in which two or more rings have a common side have a greatly increased





where M represents a metal ion Á represents a complexing agent and A—A represents a chelating agent.

stability, e g, ethylenediamine tetraacetate (EDTA) is 50 times more stable than predictions would indicate

4 Maximum stability is achieved in the presence of a minimum charge Thus inner salts are maximally stable

5 Spatial factors are important Thus primary amines are better than secondary amines which in turn are better than tertiary amines, probably because the methyl groups are cumbersome Similarly water is better than alcohol which in turn is better than ether, methanol is better than ethanol, and n propanol is better than isopropanol In addition each internal angle of a five membered ring should approximate 108° If the ring involves an aromatic nucleus and its resultant obtuse external angle no chelate is formed

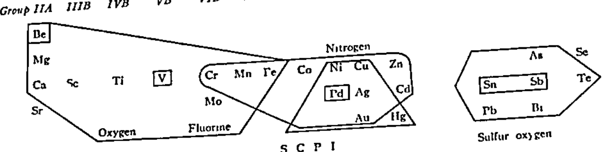
6 The active groups involved in chelation are (1) fluorine and oxygen (2) nitrogen (3) sulfur carbon (CN carbonyl) phosphorus (as phosphene) and the halogens The first group has maximum binding capacity for beryllium with a secondary one for vanadium The second group has a maximum binding for cobalt and the third for palladium and copper In addition both sulfur and oxygen bind metals centering around tin and antimony in the Periodic Table (Table XXV)

7 Transition metals are the most strongly bound because their inner shells are unfilled and allow a shifting of electrons to meet the optimum chelating requirements whereas other metals are much more rigid in the way they can accept or donate electrons

The spatial type of chelate is also of interest (Table XXVI) Beryllium and boron zinc, cadmium and mercury form tetrahedral complexes Presumably the first two could resemble magnesium chelates, the last three each other Copper silver, gold and nickel form square complexes while all of the other essential metals save molybdenum

TABLE XXV

AFFINITIES OF VARIOUS COMMON TRACE METALS FOR CHELATION BY DIFFERENT ACTIVE GROUPS



After Bailar (234)

TABLE XXVI

NORMAL AND ABNORMAL METALS IN CHELATES (233)

Coordination No	Tetrahedral	Square	Octahedral	Dodecahedral
4	Zn^{++}	Cu^{++}		
4	Cd^{++} Hg^{++}	Ag^{++} Au^{+++}		
		Ni^{++}		
6	Mg^{++}			
6	Be^{++} B^{+++}			
6			Mn^{+++} Fe^{++} Co^{++} Co^{+++}	
6			Cr^{+++}	
6			Al^{+++} Pb^{++++} Sn^{++++}	
8				Mo^{++++}
8				W^{++++}

See footnote p 193

form octahedral ones, as do chromium aluminum, tin and lead Thus possible interfering metals may be roughly grouped according to their chelate forms Those of the first transitional group have the requisite outer shell unfilled a measure of reactivity

Trace Metals and Metalloenzymes In Table XXVII is a partial list of some of the enzyme systems believed to contain a trace metal as a prosthetic group The list is by no means complete and undoubtedly will be expanded in the future as enzymes are purified According to Williams (235) and Najjar (236) there are two types In one a specific element and no other is firmly combined or chelated with the protein apoenzyme for enzymatic activity, other metals may inhibit activity The second is relatively less specific in that two or more metal ions usually in the first transitional series can be interchanged and the metal is more or less dissociable from the protein Some of the peptidases were thought to have only partial specificity until the work of Emil Smith (140) strongly

suggested that metal ions considered interchangeable such as magnesium manganese and cobalt are specific for en zyme activity and cannot be interchanged in the strict sense of the term

Certain metalloenzyme systems need organic coenzymes (or vitamins) for activity One possible example is pyri doxal phosphate which according to Snell requires a metal for the coenzyme to become activated (215) While the metal is not known model systems constructed without the apoenzyme suggest that either copper iron or alumi num could be the essential one (237) It is apparently so firmly bound that most metal binding agents will not re move it Other well known examples are riboflavin where flavin adenine nucleotide is intimately bound with the oxidation and reduction of iron and the copper flavinoid in acyl Coenzyme A-dehydrogenase Other members of the vitamin B group in some cases may require metals for activity magnesium with thiamine and molybdenum with flavin adenine nucleotide are examples

In general the active essential metals are divalent and in the first transitional group of the periodic table Copper is essential for phenolic and catecholic oxidation and for fat metabolism Manganese is required for peptide splitting and for carboxylation In view of the high concentrations of zinc in tissues and the very few zinc enzymes found carbonic anhydrase being the most prevalent it is possible that others exist The metallo-porphyrins are good ex amples of chelates heme the prosthetic group of hemo globin has iron chelated to four methyl pyrole rings the iron porphyrins of the cytochromes and myoglobin vitamin B_{12} has cobalt chelated in a porphyrin structure and the porphyrin of chlorophyll chelates magnesium There are several metalloproteins known ceruloplasmin and hemocuprein contain copper mercaptalbumins ap-

TABLE XXVII

EXAMPLES OF SOME METALLOENZYMES MOSTLY MAMMALIAN*

	Essential Metal	*Other Activators*	*Organic Co-enzyme*	*Remarks*
Copper Enzymes				
Tyrosinase	Cu^{++}		?Pantothenic acid	0 1–0 3% Cu
Ceruloplasmin	Cu^{++}			0 32% Cu
Hematocuprein	Cu^{++}			Function undiscovered
Hemocuprein	Cu^{++}			
Hemocyanins	Cu^{++}			
Acyl Co A dehydrogenase	Cu^{++}		Flavin	
Proteolytic Enzymes				
Leucine aminopeptidase	Mn^{++}	Mg^{++}		
Glycylglycine dipeptidase	Co^{++}	Mn^{++}		
Glycylleucine dipeptidase	Mn^{++}	Zn^{++}		
Prolidase	Mn^{++}			
Carboxypeptidase	Mg^{++}			
Carnosinsase	Mn^{++}	Zn^{++}		
Dehydropeptidase	Zn^{++} (?)			
Arginase	Mn^{++}	Fe^{++} Co^{++} Ni^{++}		
Polypeptidases	Zn^{++} or Co^{++}			
Keto Acid Carboxylases				
α ketoglutaric carboxylase	Mg^{++}	Mn^{++}	Diphosphothiamin	
Pyruvic acid oxidase	Mg^{++}	Mn^{++}	Diphosphothiamin	
Oxaloacetic carboxylase	Mn^{++}			
Oxalosuccinic carboxylase	Mn^{++}			
Carbonic anhydrase	Zn^{++}			0 31–0 34% Zn
Phosphatases (most)	Mg^{++}	Mn^{++} or K^{+}		
Acid	Mg^{++}			

* After Lehninger (267) and others (307 245)

TABLE XXVII—(*continued*)

Alkaline	Mg^{++}	Zn^{+} Co^{++} Ni^{++} Cd^{+}		
ATPase	Ca^{++} Mg^{++}			
Hexosediphosphatase	Mg^{+}			
Transphorylase	Mg^{++} $(+K^{+})$			Phosphoenol pyruvate to ADP
Flavokinase	Mg^{++}		Riboflavin	
Actomyosin	Ca^{++}			Inhibited by Mg^{++}
Arginine ATP transphorylase	Mg^{++}			
Phosphoglucomutase	Mg^{++}			
Hexokinase	Mg^{++}			
Enolase	Mg^{++} or Mn^{++}	Zn^{++}		
Dehydrogenases				
Phosphogluconic acid	Mg^{++}	Mn^{++}		
Isocitric	Mn^{++}			
Alcohol	Zn^{++}			
Oxidases				
Xanthine	Mo^{+VI}		FAD	0.03% Mo
Aldehyde	Mo^{+VI}		FAD	
Monamine	V^{+IV}			Only activator found†
Histaminase	?		Pyridoxal PO_4?	Metal not known
Decarboxylases				
5 hydroxytryptophan	Zn^{++}	Mn^{++} Mg^{++}	Pyridoxal PO_4	
DOPA	Zn^{++}		Pyridoxal PO_4	Metal inferred†
Histidine	?		Pyridoxal PO_4	
Oxalacetate	Mn^{++}	Co^{++}		
Others				
Transaminase	?		Pyridoxal PO_4	Metal not known
Uricase	Zn^{++}			

† See Table XXIII p. 126 and p. 154

parently transport zinc and copper by imidazole binding and gamma globulins contain various metals some possibly bound to sulfhydryl groups The discovery of hexavalent molybdenum as essential not only drew attention to heavier metals but was also the first example of a high valency metal in an enzyme Therefore, higher valency metals in the first transitional group are not excluded from essential functions (Ti V, Cr) nor are heavier ones

The practice of suspecting the presence of a trace metal in an enzyme system by attempting to inactivate it with metal binding agents (cyanide, sulfide azide, mercaptans thiocyanate, ethylenediamine tetraacetate) may lead to false assumptions if the metal is more tightly bound to the enzyme than it can be to the binding agent In the case of carbonic anhydrase a known zinc metalloprotein many strong chelating agents apparently fail to inhibit activity although some binding agents do (238) Inhibition by sulfanilamide may be the result of zinc binding Strange to say, zinc itself is a potent inhibitor as are silver gold copper mercury and vanadium The kinetics of such reactions have not been described sufficiently to allow predictions as to different metals involved purification of an enzyme now is the only real proof

POSSIBLE COMPETITIONS BETWEEN ABNORMAL AND ESSENTIAL TRACE METALS

The validity of the Periodic Table appears established for physics geology, chemistry and metallurgy but until recently there has been little application of these disciplines to the biochemistry of disease Substitution of one element of a periodic group for another, however has been often demonstrated in specific mammalian tissues

Substitution of One Element for Another In group II B radium barium strontium calcium and beryllium all

have an affinity for bone both strontium and beryllium can cause rickets and beryllium displaces magnesium on phosphatases inactivating them Therefore all but magnesium are concentrated by one tissue Similarly two anionic and all cationic elements in group VII have been shown to be concentrated by the thyroid iodine astatine manganese technetium and rhenium those of the halide sub-group quite specifically (239 242) Likewise in group V A bismuth antimony and arsenic are believed to displace phosphorus in phosphates Gold silver and copper of group I B have strong affinities for each other as does cadmium for zinc in group II B complete separation of the two from ores is difficult and often too expensive for commercial purposes Cadmium displaces zinc on human mercaptalbumin while lead does not presumably because the former two ions bind the same molecular group (the 16 imidazole groups) while the latter binds at a different site Cadmium therefore has a higher affinity than zinc for this protein Similarly cupric ion is displaced from the sulfhydryl groups of bovine serum albumin by metals in the following order of affinity $Hg^{++} > Pb^{++} > Cd^{++} > Zn^{++}$ (243) Among the anions fluorine chlorine bromine and iodine are biologically interrelated while selenium and tellurium of group VII A will displace sulfur in hair and nails possibly in sulfhydryl groups In group I A radio-rubidium is used to measure potassium space (244)

Metalloenzyme Inhibitions In spite of these obvious relationships in biological material metalloenzyme competition by an extraneous metal has not been systematically studied In Table XXVIII are shown examples of cases where an extraneous metal apparently displaces an active metallic prosthetic group The list may be far from complete At least two types of enzyme inhibition can occur

TABLE XXVIII

SOME EXAMPLES OF INHIBITION OF METALLOENZYMES BY METALS

Enzyme	Activator	Inhibitor	References	Remarks
Alkaline phosphatase*	Mg^{++}	Be^{++} Cu^{++}	298	
ATP ase*	Ca^{++} Mg^{++}	Be^{++} Cu^{++}	298 299	Other inhibitors less effective
Arginase*	Mn^{++}	Be^{++}	267	
Carbonic anhydrase	Zn^{++}	Cu^{++} Ag^{++} Au^{++} Zn^{++} Hg^{++} V^{+++}	238	Other metals (Cd^{++}) forming insoluble bicarbonates or phosphates may compete
Tyrosinase*	Cu^{++}	Au^{++} Ag^{+} Hg^{++}	246	
Leucine aminopeptidase	Mn^{++}	Cd^{++} Cu^{+} Hg^{++} Pb^{++}	300	
Carnosinase*	Zn^{++} or Mn^{++}	Ca^{++} Cd^{++}	301	
Xanthine oxidase	Mo^{+VI}	Cu^{++} Hg^{++} Ag^{+} Pb As	302	
Aldehyde oxidase	Mo^{+VI}	As	303	No other transition elements or Cd
Glutamic transferase	Mn^{++}	Cu^{++}	301	
Succinic dehydrogenase	Cu^{++} ?	Cd^{++}	238	
Choline oxidase	?	Cd^{++}	238	
Prolidase	Mn^{++}	Co^{++} Ca^{++} Zn^{++}	305	Metal not established
Glycylglycine peptidase	Co^{++}	Zn^{++}	306	
Phosphoglucomutase	Mg^{++}	Cu^{++} Ag^{+} Hg^{++} Zn^{++} Pb^{++}	238	

*Probably by specific displacement. For other examples see Tables XXIII and XXVI

One is when a heavy metal combines with sulfhydryl groups to inactivate the enzyme Mercury copper and silver will inactivate many enzymes which do not contain a metal (245) Presumably the toxic effects of many heavy metals are due to this type of reaction The second is when the essential metal is displaced by another often of the same periodic group Lerner has offered good examples of both types of reactions in respect to tyrosinase essential for formation of melanin (246) Metals which compete with copper Increased melanin pigmentation is frequently observed when heavy metals such as arsenic bismuth iron gold silver and mercury are deposited in the skin Patients with hemochromatosis have relatively large amounts of iron and copper deposited in the skin The most plausible explanation for these findings is that metals bind epidermal sulfhydryl groups and thereby re lease inhibition of tyrosinase The increased tyrosinase activity results in increased melanin formation However if sufficient quantities of the metals mercury silver or gold are present they can replace the copper of tyrosinase to produce an inactive enzyme with resultant depigmenta tion It is possible that the slight decrease in skin color produced by ammoniated mercury freckle creams is achieved in this manner Six copper binding agents have caused depigmentation *in vivo* (247) most of them anti thyroid drugs

Essential metals such as copper manganese cobalt and zinc can interact to inhibit the metalloenzymes of each Excess enzymatic activity by a presumably abnormal metal can also occur For example chromium causes increased synthesis of cholesterol and fatty acids by rat liver (248) cadmium and cobalt enhance bacterial oxalacetic carboxy lase a manganous enzyme vanadyl ion enhances mona mine oxidase (Table XXIII) Thus both stimulation and depression by abnormal metal are possible

The effects of a series of metals on two enzymes possibly concerned in arterial hypertension are shown in Table XXIII At the highest concentrations it is probable that inactivation of dihydroxyphenylalanine (DOPA) decarboxylase by metals was nonspecific or caused by sulfhydryl binding at low concentrations the specific inhibition by cadmium and mercury may be the result of displacement of another essential metal Since the coenzyme is pyridoxal probably with a metal displacement of the chelated essential metal is the most plausible explanation of the mechanism of inhibition If so zinc may be the essential one Obviously high concentrations of cadmium in the kidney might cause serious metabolic alterations in the decarboxylation of several amino acids

If interference of a single metalloenzyme system can be caused by another competing and inactivating metal certain chronic diseases could ensue As a theoretical example if the molybdenum in xanthine oxidase were replaced by another in the same periodic group for example, tungsten or chromium, and the enzyme so inhibited gout might be produced There is no evidence whatsoever that gout is due to tungsten or chromium but this is a theoretical possibility Interference by an extraneous metal such as chromium, with manganous ion in the Krebs cycle would interfere with carbohydrate metabolism Any one of these reactions could have profound and lasting results if a sizeable part of the total activity of the enzyme in the body were inhibited

Simple Chelating Compounds A great many organic compounds possess the ability to bind metals in more or less dissociable complexes Many others form chelates in which two or more electron donors combine with the metal to produce one or more rings (233) The importance of chelation has long been appreciated in industry but only

lately in biology although the calcium citrate chelate is commonly used to prevent clotting of blood All metallo-enzyme reactions are believed to depend upon chelation

Each chelating compound differs widely in its affinity for different metals In Table XXIX are shown the stability constants of a number of common oxygen and nitrogen chelators with divalent metals of the first transitional group and with cadmium Ten of these compounds are present in biological fluids In general an increase in tightness of structure is proportional to atomic number reaching a peak at copper (or nickel) and decreasing thereafter This fundamental property of most chelating agents must be in mind whenever they are used, in effect this property means that a free ion having a higher stability constant with the chelator will displace a chelated metal with a lower constant When the active groups are sulfur and other chelators the pattern of metallic affinity is different (Table XXV)

Metal Binding Simple metal binding is dependent either upon the tightness of the bond the lack of dissociation of the dissolved salt or upon the insolubility of the complex Thus complexes may be formed between metal and ammonia metal and sulfhydryl metal and cyanide or metal and hydroxide The stability constants for transitional metal complexes in solution are usually lower than for chelates although mercury has a fairly high affinity for CN NH_3 OH and pyridine The common law relating the stabilities of chelates of the first transitional group and atomic numbers appears to operate in the case of simple complexes of OH CN NH_3 and pyridine

DRUGS AS CHELATING AGENTS

Most potent drugs may act through their abilities to chelate trace metals on metalloenzymes Schubert states

(232) The action of many drugs probably can be explained, partly or wholly, by their possession of groupings capable of binding metals The drug whether deleterious or helpful to the organism inhibits or activates a physiological function in which a metal is required Inhibition or activation may come about in several ways

'1 The drug may chelate a metal ion needed for the activation or inhibition of an enzyme system and hence, indirectly necessary for survival

2 The drug by its chelating action may facilitate the transport of a metal ion to its site of action, in some cases by the formation of a fat soluble metal chelate

3 The chelating drugs may render available for metabolic purposes a metal ion which otherwise might remain in an inactive, insoluble form

4 The metal chelate may be readily excreted, thus providing a means of detoxification

'Examples of drugs which may act through chelation by some of these actions are given below Of further interest are the factors determining whether or not a metal chelating drug will be effective, since these same factors are responsible for chemical specificity in biological systems Knowledge that metal chelation is the basis of a drug s action facilitates considerably the development of new drugs because it becomes possible to anticipate how the drug molecule can be modified to enhance its metal binding properties

In the case of salicylic acid it is highly probable that metal chelation is important This is shown by the fact that the m and p hydroxybenzoic acids which do not chelate are inactive as analgesic agents or against the fever and pain associated with rheumatic fever It is not known which metal or metals are affected by salicylic acid, but it is known that the metals affected must belong to the

transition groups Numerous derivatives of salicylic acid containing the o-carboxyl hydroxyl group are about as effective as salicylic acid although they differ in dosage required and side effects It might be anticipated that physiologically cortisone would have some resemblance to salicylates as has been demonstrated experimentally Terramycin and aureomycin reverse the Be inhibition of alkaline phosphatase through the formation of a complex ion with Be He lists as examples salicylic acid adrenalin terramycin aureomycin a thiosemicarbazone and corti sone Penicillin forms insoluble salts with heavy metals

Many chelating agents are fungicidal antiseptic or bac tericidal (233) Thirteen of nineteen common organic chelators of one or more transitional metals are listed by Martell and Calvin as effective against growth of B subtilis with seven against growth of B Coli Likewise 8-hydroxy quinoline of seven quinolines and 26 substituted *quinolines* of 35 are effective against the growth of clostridium welchii most of which inhibit streptococcus hemolyticus staphylococcus aureus and B Coli less so proteus and pseudomonas pyocyaneus Zinc and manganese appear to be bound although the other essential metals iron and cobalt *may* be involved Apparently copper is not for copper reagents in general are inert Few are effective against pyocyaneus an *organism resistant* to most anti biotics The fungicidal properties of the *oxines* so widely used *in industry* is believed to be *the result* of their chelation with zinc an essential metal for growth There fore bactericidal and fungicidal activity of many antiseptics and antibiotics may be functions of metal binding or chelation

The most popular and *versatile* of the recognized chelat ing drugs is ethylene-diamine tetraacetate (EDTA, Seques trene Versene) stability constants for which are shown in

TABLE XXIX

THE LOGARITHM OF THE STABILITY CONSTANTS OF SOME METAL COMPLEXES*

Ligand	N	log K_N Mg	Ca	Mn	Fe	Co	Ni	Cu	Zn	Cd
NH CH CH NH (En)	2	Small	Small	4 8	7 5	10 7	13 8	19 6	10 4	10 0
Histidine	2	—	—	7 7	—	13 8		18 6	12 8	—
N(CH CH NH) (Tren)	1	—	—	5 8	8 8	12 8	14 8	18 8	14 6	12 3
CH NH(CH CH NH) CH NH(CH CH NH) (Trien)	1	—	—	4 9	7 8	11 0	14 0	20 4	12 1	10 7
Glycine	2	6 5	1 4	6 6	8 0	8 9	11 1	15 4	9 7	8 6
Glycyl glycine	1	1 1	1 2	—	—	3 5	4 5	6 0	3 8	—
N(CH CO H) (Triac)	1	5 4	6 4	7 4	8 8	10 6	11 3	12 7	10 4	9 5
CH N(CH CO H) CH N(CH CO H) (EDTA)	1	8 7	10 6	13 5	14 2	16 1	18 5	18 4	16 1	16 5
Oxalate	1	3 4	3 0	3 9	—	4 7	5 3	6 2	4 9	3 9
Tartrate	1	1 4	1 8	—	—	—	—	—	2 8	—
Sal cylaldehyde 5 Sulphonic acid	2	—	—	—	—	5 6	6 6	9 3	5 4	—
Salicylaldehyde†	2	6 8	—	6 8	7 6	8 3	9 2	13 3	8 1	7 8
Acetyl acetone†	2	9 5	—	—	—	11 2	12 1	17 1	—	—
Polyphosphate (N =2)	?	3 2	3 0	2 5	3 0	3 0	3 0	3 5	2 5	—
Hydroxyl ion	1	2 6	1 1	2 8	3 2	3 6	3 8	6 5	4 7	3 0
Dipyridyl	3	—	—	—	16 5	—	—	17 8	—	10 5
ortho-Phenanthroline	3	—	—	—	21 5	—	—	—	17 0	15 2
Riboflavin	?	—	—	3 4	7 1	3 9	4 1	6 6	5 6	4 7
Folic Acid	?	—	—	6 0?	7 9	8 1	9 0	7 8	7 5	6 7

* After Williams (235)

† The measurement of these stability constants was made in a mixed dioxan/water solvent whereas the other m asu em nt wer all m de In aqueous solut on

Table XXIX In addition to the transitional metals many others have strong affinities lead (18 2) lanthanum (15 4) chromic (24 0) ferric (25 0) as well as beryllium vanadium titanium silver and many rare earths * Because EDTA is not metabolized (249) and apparently enters cells (250) it provides a means for removing soluble ions from the body in the order of their stability constants If an abnormal trace metal is to be removed normal ones will accompany it according to the relative amounts and stability constants of each Thus EDTA is not specific for lead for example a current popular use but will remove other ions with a higher constant such as copper and nickel and especially ferric iron EDTA will be inactive however if the metals in the body are more tightly bound or chelated to protein than to the drug This relative chelating capacity of a sequestering agent and a metal in the body follows certain definite laws and explains the ineffectiveness of EDTA for removing most metals

An example of the effects of EDTA given intravenously to two patients is shown in Table XXX Zinc was removed in sizeable quantities other metals less so or not at all The high excretion of zinc in the patient with the nephrotic syndrome found before the drug was given is probably explained by the excessive proteinuria which carries combined zinc There was no mobilization of lead while

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EDTA makes a good chelating agent for clinical use *for the following reasons* 1 The stability constants (Log K_s) for common but *important* loosely bound metals is low (Ba Sr Ca Mg = 7 76-10 96) 2 The constants for more tightly bound metals is moderate (Mn Fe^{++} *Co Cu* Zn = 14.04 18.8) 3 The constants for several abnormal metals is high (Hg 21.8 V^{+++} 25 9 Fe^{+++} 25 1) Unfortunately *several* abnormal *metals* fall *in* the range of the essential ones (Pb 18 04 Al 16 13 Ni 18.6? Y 18.09 Cd 16 46) so that these cannot be removed without danger *of* essential metal deficiency notably zinc.

RENAL EXCRETION OF 13 METALS BEFORE DURING AND AFTER INTRAVENOUS EDTA

Date	I V EDTA gm	Urine Volume l	Urine Protein gm/l	Ti	V	Cr	Mn	Ni	Zn	Mo	Ag	Cd	Pb	Sn	Fe*	Cu*
				Parts (by weight) per billion parts of urine												
Normal Subjects		Mean		<3.8	<0.63	<0.64	<9.7	<2.8	<67	<14	0.80	<0.86	<5.7	<3.2		
Nephrosis																
11/ 3/54	0	1.55	—	2.0	3.1	0.17	13	3.2	360	5.0	0.74	2.9	0.80	13	570	330
2/25/55	0	2.66	2.6	0.37	<1.0	0.14	<5.0	0.75	360	3.6	0.59	1.3	3.4	22	560	—
2/26/55	0	2.15	2.4	0.16	<1.0	<0.05	<5.0	1.8	170	3.5	0.76	0.88	0.28	6.0	560	162
2/27/55	0	1.80	2.2	0.88	<1.0	<0.05	<5.0	2.2	370	3.2	0.54	2.5	0.60	14	660	230
2/28/55	0	2.36	1.6	0.95	<1.0	<0.05	<5.0	2.2	240	4.1	0.57	1.4	0.22	6.4	460	265
Mean	0	2.24	2.2	0.59	<1.0	<0.07	<5.0	1.7	285	3.6	0.62	1.5	1.1	12	560	219
3/ 1/55	3	1.81	2.4	1.1	<1.0	0.11	21	2.4	1900	3.0	0.36	<0.5	0.27	2.5	1050	180
3/ 2/55	3	2.01	2.8	2.0	<1.0	0.35	12	4.0	1400	2.2	0.32	<0.5	0.07	2.2	1070	162
3/ 3/55	4	1.46	3.2	0.50	<1.0	7.3	15	16	2200	2.4	0.38	2.8	0.70	4.8	1930	187
3/ 4/55	0	1.97	2.4	0.73	<1.0	0.12	8.5	2.4	1200	1.2	0.37	4.0	0.04	3.4	1450	385
Mean	2.5	1.81	2.7	1.1	<1.0	2.0	14	6.2	1675	2.2	0.36	1.9	0.27	3.2	1375	229
3/ 5/55	0	1.73	3.2	1.3	<1.0	<0.05	<5.0	3.0	640	<0.5	0.43	3.6	0.13	3.9	—	385
Normal Female	39															
10/ 6/55	0			2.7	7.9	6.75	<6.0	1.15	17	7.0	0.4	<1.02	70.1	10.25		
10/ 8/55	0			—	1.1	1.8	<5.0	2.9	25	16	0.51	<0.5	2.0	14.25		
10/11/55	0			1.8	7.4	4.0	Tc	1.75	50	13.25	0.34	0.85	215	85		
Mean				1.5	5.5	4.2	±4	1.9	31	12.08	0.42	±0.79	95.7	36.5		
10/16/55	3			0.65	<0.5	0.78	Tc	3.1	46	12	0.33	0.68	7.0	13		
10/17/55	3			16	2.35	1.45	16	2.0	490	9.25	0.43	0.9	26.5	3.0		
10/18/55	3			8.25	1.8	1.75	17.5	1.75	650	9.25	0.43	0.58	55	5.5		
10/20/55	3			5.0	1.55	1.5	27	1.6	800	13.5	0.43	14	87.5	5.25		
Mean				7.46	±1.55	1.37	±15.1	2.11	496.5	11.0	0.41	4.04	44.0	6.69		

From data of Perry and Schroeder (181) The normal ubjects we e 15 labo atory wo k s Note the in rease in h omium manga ese nl k l nd i on in the n phrotic p t nt in titan um manganese and cadm um n th normal wom n and the no mou l crease n nc in both ubject Th lim ti g factor of th s d ug i obviously z nc defi ency Note also th fa lu of lead to move The high i ti l x tio of i c nd ppe in the ph oti p ti t represented p t n bound metal n urin

The i on det m nations w don by D Reubenia S Dubach and th copper by Dr Clark J Gubler

eight metals almost certainly present in a strongly bound form in their tissues did not change

BAL (2 3-dimercaptopropanol) a straight chain dithiol binds the following heavy metals in a chelate zinc chromium cadmium nickel, lead, antimony, arsenic, bismuth copper, mercury, gold (251) Substitution complexes on the sulfhydryls are formed In the case of cadmium at least, these are dissociated in the kidney and may result in cadmium nephritis a reflection of the greater binding capacity of renal tissue for cadmium than BAL. Citrate a chelating agent used for lead poisoning is metabolized by the body and is therefore relatively ineffective A list of some representative binding and chelating agents is shown in Table XXXI Their use in medicine is only beginning For example all antithyroid drugs have this common property suggesting their probable action

These considerations open up a wide field of thought on the mechanisms of disease and of drug actions Similar conclusions can be drawn when late toxic reactions of drugs are compared with structure The common denominator of the offending drugs appeared to be in chelation

Drug *Reactions* Late systemic reactions to drugs affect several organs and systems of which blood dyscrasias hepatitis and polyarteritis are the most serious (252) Most of the drugs causing fatal agranulocytosis as listed by Alexander (252) are metal binding agents (233 253) such as aminopyrine phenylbutazone antihistamines dinitrophenol Presidon procaine amide and Tapazole contain ing pyridines amines amides nitroso or sulfhydryl groups (253) Their solubilities and specificities for heavy metals however are not known to our knowledge Nonfatal leucopenia has occurred with arsenical compounds gold salts thiouracils hydantoins salicylates sulfonamides streptomycin and thiosemicarbazone which can displace or bind

TABLE XXXI

SOME EXAMPLES OF CHELATING AND METAL-BINDING DRUGS (232 233 253)
(OTHER THAN ANTIHYPERTENSIVE AGENTS)

	Reference
Drugs Used To Remove Metals	
EDTA	233
BAL	251
Citric Acid	235
Antithyroid Agents†	294
Thiourea	247
Thiouracil	247
2 Mercaptoimidazole* (Tapazole)	253
2 Aminothiazole*	253
P Aminobenzoic acid*	253
Sulfonamides	233
L-5 vinyl 2 thio-oxazolidone	294
Antibiotics†	
Sulfonamides	233
Penicillin (and penicillamine)	253
Chloramphenicol*	253
Streptomycin*	253
Isoniazid	296
Thiosemicarbazone*	253
P Aminosalicylic acid	233
Analgesics and Antipyretics†	
Aminopyrene	233
Antipyrine	253
Phenylbutazone*	253
Phenacetylurea*	253
Salicylic acid	233
Miscellaneous†	
Bis(diethylthiocarbamyl) disulfide (antabuse)	297
2 Acetylamino 1 3 4 thiadiazole 5 sulfonamide (Diamox)	233
Drugs Containing Specific Groups†	232 233 234
Pyridine	
Thiol or Mercaptan	
Carbazide	
Diazine	
Thiazol	

* Contains metal binding groups affinities not known

† Drugs causing late toxic reactions

TABLE XXXI—*(continued)*

Nitrite	
Thiocyanate	
Pyrocatechol	
Quinaldine	
Hydantoin	
Hydrazine	
Chelating Chemicals *	
Phytic Acid	
2 ketogluconic acid	
Glycerophosphates	
Potassium gluconate	
Gallates	
Rubeanic acid and derivatives	
Guanidine carbonate	
Potassium ethyl xanthate	
Dimethyl glyoxine	
Uracils	
Oximes	
Diphenyl carbazide	
Diphenyl thiocarbazone	
Potassium thiocarbonate	
Cupferron	
Adipoin	
Some Reagents for Analysis of Metals	256
Zn Ferric cyanide	
8-hydroxyquinoline	
Quinaldinate	
Sn Dinitro-diphenylamine sulfoxide	
Toluene dithiol	
Ag P-dimethylamino-benzalrhodamine	
Ni γ benzil-dioxime	
Dithiooxalate	
Dithiol	
Mo Thiocyanate	
Cu Quinosol	
Pyridine thiocyanate	
Cd β-napthoquinoline	
Thiourea	
Benzoin-oxime	

* From chemical catalogues

trace metals The properties of the few other drugs causing this disorder are not known Even the more unusual cases of leucopenia are due to such metal binding agents as barbiturates, chloramphenicol, isoniazid, demerol phenothiazines novalgin, pamaquine, penicillin and phenurone, which appear to possess the requisite metal binding groups or to form insoluble metallic salts

Alexander s list of drugs reported to cause aplastic anemia contains eight of the above agents, with the addition of such possible metal binding agents as quinacrine hydralazine, mercurial diuretics (both mercury and amide are present in mercuhydrin) novurone and para aminosalicylic acid The drugs causing thrombocytopenic purpura include eleven of the above with the addition of sedormid procaine quinine and quinidine containing either similar groups or quinoline In the case of quinine and quinidine the quinoline structure is not such as to have chelating properties the requisite group being on the 4 position while 8 hydroxyquinoline is a strong chelating agent Unless the ring structure is broken in the body to form quinolinic acid the mechanism of action is probably not dependent upon chelation Hepatitis has been caused by 17 of the above drugs and by acriflavine, cortisone and carbazone all of which contain metal complexing or sequestering groups

Aside from proteins and undefined extracts of plant and animal tissues many of the same drugs appear in the list of causes of what Alexander calls the serum sickness pattern (252) Twenty one of the above drugs are listed, with the addition of thiocyanate ACTH (which may mobilize zinc and contain sulfhydryl) bismuth, two new antibiotics structurally unidentified and chlorpromazine, which obviously has metal binding properties when hydrolyzed When a comparison is made with the substances

causing shock 13 new ones appear which do not have this property and 13 of the above are represented Bronchial asthma caused by 36 agents of which 19 are of plant or animal origin and unidentified structurally is caused by 14 containing possible metal complexing groups or metals A similar situation appears among the agents causing severe late toxic skin reactions such as eczema urticaria exanthemata exfoliative dermatitis bullous eruptions and the like Metals and binding agents appear frequently when the chemical structure is known

Curiously enough serious local and systemic reactions are rare or absent among the drugs not containing metal reactive groups or producing them only on extensive hydrolysis Sulfobromphthalein decholin paredrine *ether boric acid* Banthine menthol quotane diocaine chloral hydrate morphine opium codeine digitalis are examples On the other hand barbituric acid the basic constituent of many sedatives and a pyridine compound forms salts with metals Metal binding by sulfanilic acid is well known (233) The instability of hydantoin hydrolyzing to metal binding hydantoic acid the metal binding properties of pyridines nitroso groups (dinitrophenol is a good example) cyanides amine and sulfhydryl groups semicarbazides dicarboxylic acids thiols and sulfur-containing structures appear to be related to many forms of drug sensitivity Therefore it is possible that trace metals may be involved in many reactions of sensitization and perhaps even in some forms of allergy BAL (2 3-dimercaptopropanol) for example is a potent contactant Although drug reactions vary widely in frequency such agents as dinitrophenol being very active and amines relatively inactive metalloenzyme disturbances cannot be excluded as causes It will be noted that the more metal binding groups on the soluble sulfonamides (pyridine thiazole succinate etc.) the more likely

the agent is to produce late generalized reactions The interested reader can find many examples of this apparently general phenomenon by comparing structure (253), metal binding power (233) and ability to produce late reactions (252)

The author by no means wishes to imply that the phenomena of hypersensitivity, allergic and immune reactions, and anaphylactic shock are the result of trace metal chelation The mechanisms of these reactions involving altered proteins have been studied extensively enough to exclude trace metals as being primarily responsible Late toxic reactions to drugs, however may well be caused either by metal imbalances or sensitivity Thrombocytopenia induced by quinine is an example of the latter Obviously the problem should be studied further from this viewpoint

ESSENTIAL TRACE METALS IN MAN

To be essential for mammalian metabolism the trace metals under consideration should be able to form chelates and to be reactive They should be demonstrable in human tissues both of infants and of adults They should be found in sea water, for life began in the sea and in plants They should demonstrate activity on enzyme mechanisms especially to accelerate reactions We can apply these criteria to the metals actually found, examining the most logical ones (Table XXXII)

In Table XXXIII are shown the metals of interest found in sea water In general those of highest concentration are more prevalent in plants and animal tissues The relative composition of sea water however has probably changed since life began in the primitive ocean only those elements present when amphibians left the ocean

TABLE XXXII

TRACE METALS FOUND IN MAN AND THEIR PROBABLE ROLES

Essential	*Possibly Essential*	*No Known Metabolic Function*	*Metabolic or Antimetabolic*	*Not Found But Antimetabolic*
Cobalt	Aluminum	Barium	Bismuth	Antimony
Copper	Strontium	Boron	*Cadmium*	Arsenic
Iron	Vanadium	Cesium	*Chromium*	Beryllium
Manganese		Gallium	Gold	Thallium
Molybdenum		Lanthanum	*Lead*	
			Nickel	
Zinc			Silver	
		Tin	*Titanium*	

Those in italics may be implicated in chronic diseases especially cardiovascular because of their prevalence concentrations or known functions on enzyme systems.

and satisfying certain criteria for reactivity in enzyme mechanisms can be expected to have become essential for metabolism We cannot say that living cells have learned to use new and less reactive elements in enzyme systems by a process of adaptation the basic structures of atoms have not changed and life began by using the most suitable ones

Because all food comes eventually from plants an examination of the metallic content of plants is necessary local pastural variations can be neglected No metal can be expected to be essential for animals which does not occur in plants or in water In Table XXXIII are the metals of interest in plants Little aluminum nickel and no cadmium tin silver gold titanium lead or mercury is to be expected in animal tissues while vanadium and the five known essential metals will be found Obviously if domestic animals or man show appreciable quantities of those which do not appear in plants they must have come from unnatural sources

To be classed as abnormal for our thesis a metal

TABLE XXXIII

TRACE METALS IN SEA WATER %*

(Also found in plants animals and man (256 257 231 254))

	Seawater		Essential for Plants	Found in American Tissues			Essential for Mammals	Possible Contaminant	
	Factor	$\times 10^{-}$		Infants	Adults	Adult Urine		from Soil	from Civilization
Mg	1 3	1	+	+	+	+	+		
Sr	1 3	3	?	+	+		+?		
B	4 5	4	+	+	±		?		
Rb	2 0	5	?	?	+				
Zn	5 0	6	+	+	+	+	+		
Fe	5 0	6	+	+	+	+	+		
Cu	2 0	6	+	+	+	+	+		
Al	5	5	0	+	+	?	?	+	+
Pb	5 0	7	0	+	+	+	0		+
Mn	4 0	7	+	+	+	+	+		
Ni	3 0	7	0	±	±	+	0	+	+
Sn	3 0	7	0	+	+	+	0	+	+
Co	1 0	7	+?	+	+		+		
Mo	1 0	7	+	+	+	+	+		
Ti	<1 0	7	0	0	+	+	0?	+	
V	5 0	8	+?		+	+	+?		
Hg	3 0	9	0		+		0		+
Ag	n	8	0	+	+	+	0		+
Au	4 0	10	0	0	±		0		+
Cd	Trace		0	0	+	+	0		+
Cr	Trace		0	+	+	+	0	+	+

0 = Strong evidence against element being essential ? = Cannot be excluded at present

* After Vinogradov (255)

a) should be found in human tissues from some areas of the world and not from others b) should not be found in plants or wild animals c) should affect some metalloenzymes d) preferably should not be in the tissues of young infants e) should be introduced by the habits of Western Civilization into foods or beverages as a result of processing transportation or manufacture and f) should be poorly excreted cumulative and preferably showing organ specificity

Concentrations of the Essential Trace Metals in Man Although many analyses have been done by various methods for single or several elements in blood tissues and urine (254 257) the first extensive systematic investigation on the content of both essential and abnormal trace metals in human tissues was made by Tipton and her co workers Using spark spectrographic methods with indium as an internal standard and densitometric photoelectric recording of plates Tipton analyzed 258 tissues from 24 persons dying suddenly in various areas of the United States for 18 metals (almost 4500 analyses) (231) A preliminary analysis of 42 autopsies from various places in this country showed similar but less quantitative results (258) while in a later series the findings were essentially the same (259)

In Table XXXIV are the mean concentrations in various tissues of the essential elements manganese cobalt copper zinc and molybdenum calculated roughly for total bodily amounts Zinc is the most prevalent of the normal group in several times the concentration of any of the others Essential cobalt was found in only five bodies sparsely scattered in small concentrations probably because of methodological limits and its presence in very minute quantities These results appear comparable to those of Griffith *et al* for copper zinc and manganese on

TABLE XXIV

[illegible]

Organ	Weight Kg	Ni mg/Kg	Ni Total	Cu mg/Kg	Cu Total	Zn mg/Kg	Zn Total	Mn mg/Kg	Mn Total	Cr mg/Kg	Cr Total	No
Aorta	0 02	0 24	0 005	1 7	0 03	18 1	0 4	0 18	0 004			17
Brain		0 17		5 25		10 3		0 34				9
Bladder	0 02	0 24	0 05	1 40	0 18	16 6	3 3	0 12	0 03			4
Brain Cord	7 1	0 45	0 8	10 6	15 0	12 8	21 4	(0 21)	0 44			7
Heart	0 3	0 3	0 01	4 10	1 2	43 3	15 0	0 21	0 06			15
Stomach & [illegible]												11
Intestine	2 0	0 32	0 65	3 0	6 0	23 2	46 4	0 15	0 3			22
Kidney	0 3	0 4	0 1	2 77	0 83	67 2	20 1	0 63	0 2	0 20	0 06	24
Liver	1 5	1 13	1 6	10 6	15 9	68 4	102 6	1 62	2 4	0 8	1 2	25
Lung	0 7	0 6	0 42	4 5	3 2	18 1	12 6	(0 18)	0 12			23
Muscle	28 0	0 14	3 92	1 51	42 3	70 0	1960 0	(0 41)	11 5	0 24	6 75	22
Pancreas	0 1	0 74	0 07	3 0	0 3	41 0	4 1	(0 21)	0 02	0 22	0 02	22
Prostate	0 05	0 24	0 01	4 73	0 02	155 0	7 8	(0 37)	0 02			8
Spleen	0 2	0 52	0 1	8 7	1 74	22 3	4 6	(0 17)	0 03			24
Testis	0 02	0 17	0 004	2 1	0 04	13 8	0 27	(0 12)	0 02			13
Thyroid	0 03	0 48	0 013	3 5	0 1	36 6	1 1	—	—			9
Skin	4 4											
Skeleton	19 0	0 41	4 92	4 6	55 2	43 8	525 6	0 27	3 24	0 3	3 6	
Adipose Tissue	7 8											
Other Tissue	7 8											
Sub Total known (mg)			5 362		95 93		435 37		15 134		8 03	
Total with [illegible] X12 Kg (mg)			10 682		149 13		960 97		18 374		11 4	
Specimens lacking [illegible]		0		0		0		56		234		

Figures in parentheses indicate [illegible] in two or more samples. Mn, Cu, Zn were present in every sample. Mo was also [illegible] in aorta and bladder 3 [illegible]

For 70 Kg man according to [illegible] (293)

[illegible] of 24 bodies

the basis of dry tissue in a much larger series (260)

Some organs concentrate certain metals others do not Zinc was found in every organ in concentrations of 4 4 to 300 mg /Kg being far highest in prostate then muscle liver kidney and heart and lowest in adrenal bladder brain testis lung and intestine Copper was concentrated in brain liver and spleen being lowest in muscle adrenal aorta intestine and testis Manganese in much smaller amounts was concentrated especially in liver with pancreas lung spleen and thyroid following quite far behind lowest organs were heart muscle testis and aorta Three of these four metals were found in all 258 specimens examined Molybdenum was absent in only half of the samples of muscle and testis a third of those of pancreas thyroid and lung most prostates and all but one brain but was found in concentrations of more than 0 13 mg /Kg in all heart kidney and liver samples being by far the highest in liver In the concentrations detectable, molybdenum apparently is not essential for metabolism in all organs but three of the others may be

Sources and Turnover of Essential Metals All of the essential trace metals are found to a greater or less extent in plant and animal tissues (Table XXXIV) derived from the soil Cobalt is probably the least concentrated Soils vary widely in their contents of trace elements and disorders due to deficiencies or excesses have been recognized in both plants and animals Semi-quantitative trace mineral analysis is a recognized practice in the evaluation of soils for farming The following was summarized from Monier Williams (256) and Marston (261)

Manganese The largest sources are in the following foods with above 30 mg /Kg oatmeal whole meal flour bran soy bean meal cocoa cloves chestnuts pepper maple sugar tea contains 150 to 900 mg /Kg With lesser con

TABLE XXXIV

CONCENTRATIONS AND CONTENTS OF ESSENTIAL TRACE METALS IN HUMAN TISSUES (WET WEIGHT) (231)

Organ	Weight* Kg	Mn mg/Kg	Mn Total	Cu mg/Kg	Cu Total	Zn mg/Kg	Zn Total	Mo mg/Kg	Mo Total	Co† mg/Kg	Co† Total	No
Adrenal	0 02	0 4	0 005	1 7	0 03	15 3	0 3	0 18	0 004			17
Aorta		0 19		1 96		20 2		0 53				5
Bladder	0 02	0 25	0 05	3 40	0 68	16 6	3 3	0 12	0 02			4
Brain & Cord	2 1	0 40	0 8	10 6	21 0	16 8	21 4	(0 21)	0 44			7
Heart	0 3	0 1	0 03	4 09	1 2	49 2	15 0	0 23	0 06			23
Stomach & Intestine	2 0	0 32	0 64	3 0	6 0	23 2	46 4	0 15	0 3			11
Kidney	0 3	0 4	0 1	2 6	0 82	67 2	0 1	0 63	0 2	0 20	0 06	22
Liver	1 5	1 13	1 6	10 6	15 9	68 4	10 6	1 62	2 4	0 8	1 2	24
Lung	0 7	0 6	0 42	4 5	3 2	18 3	12 6	(0 18)	0 12			24
Muscle	28 0	0 14	3 92	1 53	42 9	70 0	196 0	(0 41)	11 5	0 24	6 75	23
Pancreas	0 1	0 74	0 07	3 0	0 3	41 0	4 1	(0 21)	0 02	0 2	0 02	22
Prostate	0 03	0 24	0 01	4 79	0 02	155 0	7 8	(0 36)	0 02			22
Spleen	0 2	0 5	0 1	8 7	1 74	22 3	4 4	(0 17)	0 03			8
Testis	0 02	0 19	0 004	2 1	0 04	13 8	0 27	(0 12)	0 02			24
Thyroid	0 03	0 48	0 015	3 5	0 1	36 6	1 1	—	—			13
Skin	4 4											9
Skeleton	19 0											
Adipose Tissue	7 8	0 41	4 92	4 6	55 2	43 8	525 6	0 27	3 24	0 3	3 6	
Other Tissue	7 8											
Sub Total known (mg)			5 762		93 93		435 37		15 134		8 03	
Total with estimated skin and other soft tissue at mean value X12 Kg (mg)			10 68		149 13		960 97		18 374		11 6	
Specimens lacking metal			0		0		0		56		234	

Figures in parentheses indicate element missing in two or more samples Mn Cu Zn were present in every sample Mo was absent in one aorta and bladder 3 stomachs

* For 70 Kg man according to Forbes (293)

† In only 5 of 24 bodies

may also be important in hemoglobin formation A copper enzyme ceruloplasmin is found in concentrations of about 34 mg per 100 ml of serum Several phenolic oxidases depend upon copper pigmentation may be related to the content in skin and hair follicles Copper is quite well retained by the body with some dependency upon intake being slowly eliminated by way of the feces Gallstones contain large amounts No chronic copper poisoning has been described in human beings Human milk is extremely low in content 0 04 mg /Kg As a rule American foods contain adequate amounts sometimes an excess since many insecticides and fungicides contain copper The largest amounts 10 mg /Kg or more are found in tea coffee cocoa chocolates nuts liver shell fish especially oysters tomatoes and yeast The least is found in milk butter cheese refined sugar honey margarine lard and suet

Zinc This most prevalent of the trace metals in the human body is present in large quantities in most organs as seen in Table XXXIV It is found in foods and is a requirement of plants bacteria and fungi Most modern fungicides are zinc chelating agents Deficiency in soils causes diseases of both plants and animals although its prevalence makes animal diseases more uncommon Foods with over 50 mg /Kg are wheat germ bran oysters beef livers gelatin and dried eggs those which contain the least amount are fruits chestnuts green vegetables and fish Chronic poisoning in man is *not known* There have been several outbreaks of supposedly acute poisoning from foods stored in zinc lined receptacles but the contamination of zinc by cadmium makes it questionable that zinc itself was the cause symptoms of zinc and cadmium poisoning are identical i e violent gastroenteritis and zinc poisoning is most difficult to produce in animals

centrations are green vegetables, nuts, rice and barley of considerably less content are meats, legumes and many organ tissues Dairy products and fish are low in manganese and coffee contains extremely little It has been estimated that about half the intake of adults in Britain during the winter came from tea The minimum daily requirement for man has been variously set at 4 6 to 10 mg, about 0 01 mg per day being excreted in the urine In view of the small body pool approximately 11 mg according to the method of analysis (Table XXXIV), this high requirement may reflect the poor absorption of manganese from the gastrointestinal tract Large amounts may be given daily, up to 1 0 Gm per day of the citrate or glycerophosphate, without signs of toxicity and apparently without excessive absorption While deficiency in man has not been described a reasonable assumption that it might occur can be postulated especially if conditioned by competing metals or affected by drugs Toxicity from ingested manganese has not been described although inhaled dust can cause Parkinsonian symptoms and occasionally cirrhosis of the liver

Cobalt Present in vitamin B_{12} as a porphyrin chelate, this element is essential for maturation of red blood cells but has no other known function in man It is rapidly excreted both in urine and feces and apparently does not accumulate It may not be readily absorbed in man An excessive amount produces polycythemia in the rat dog frog mouse guinea pig and sheep While the amounts in food are not well known traces occur in vegetables fruits and cereals 0 5 mg /Kg in legumes and as little as 0 003 mg /Kg in white flour In view of the small body pool deficiency in man is possible but unproven Cobalt is a vasodilator in man (262) and in the rat (183)

Copper An essential element in cell respiration copper

high content of iron in their livers Apparently the bacteria in the rumen synthesize vitamin B_{12} when cobalt is present high requirements are indicated by the large doses of the vitamin necessary to cure this disease Horses and pigs raised in the same deficient areas are not affected as their requirements are lower Bacterial synthesis in the colon, as occurs in man apparently does not lead to absorption of this vitamin, therefore it must be ingested as such *Copper* deficiency has been implicated in certain anemias in infancy but no known diseases in adults have been described Anemia has been produced in laboratory animals along with a slow rate of growth impaired absorption of ingested iron impaired mobilization of iron from tissues and impaired utilization of iron for hemoglobin synthesis have been found, as well as low cytochrome oxidase activity of the bone marrow Cattle grazing on copper-deficient pastures show depigmented, abnormal hair develop cachexia anorexia and anemia their bones become fragile reproduction and milk production is decreased and they frequently die of cardiac failure young animals may become ataxic Sheep show defective keratinization and hypochromic anemia lambs born of copper deficient ewes develop swayback and ataxic and paralytic diseases characterized by diffuse demyelinization of the central nervous system Depigmentation has been produced in many species Excesses or deficiencies of other trace elements may influence the disorders in cattle and sheep especially of cobalt and molybdenum *Molybdenum* deficiency prevents fixation of nitrogen by soil bacteria but diseases in higher animals have not been discovered *Zinc* deficiency causes hyperkeratosis in pigs (264) hyperkeratosis and keratinization of the esophagus in rats (265) sterility (256) and signs suggestive of ariboflavinosis with vascularization of the cornea and lesions at the mucocutane

Molybdenum The newest of the essential elements to be found necessary in human and animal metabolism molybdenum plus a flavin are necessary for the metabolism of xanthine to uric acid by xanthine oxidase and the oxidation of aldehydes by liver It is essential for the fixation of nitrogen in the soil by nitrogen fixing bacteria Molybdenum is present almost universally in fertile soils and in plant and animal tissues Its toxicity resembles that of selenium poisoning excesses in soils affect ruminants rather than horses and pigs, producing a disease characterized by chronic diarrhea called teart Copper fed to animals prevents molybdenum poisoning An examination of the periodic table indicates that molybdenum is unique in that it is next to the heaviest element essential for mammalian metabolism and occurs in the hexavalent form Whether or not deficiencies occur in man is not known, the body pool is small

Specific Metal Deficiences (256, 261) * Actual deficiences of some metals in man are not inconceivable, although no true deficiency (other than iron) has been described Lack of manganese kills rabbits deficiency in rats causes failure of male reproduction and a high mortality rate in the young Hen s eggs do not hatch well, perosis or 'slipped tendon with enlargement and malformation of the tibio metatarsal joint and arrested growth of long bones, possibly due to low bone phosphatase, is caused by deficiency of this element in growing chicks Pigs and rabbits also show bone affections suggesting that it may be related to the growth or health of bone and joints

Cobalt deficiency in soils causes enzootic marasmus or Bush Sickness in ruminants characterized by impaired growth anorexia, weakness emaciation and anemia with

* The reader is referred to Moore s recent critical and inclusive discussion of this subject (263)

TABLE XXXV

CONCENTRATIONS AND CONTENTS OF ABNORMAL TRACE METALS WITH ORGAN SPECIFICITIES (WET WEIGHT) (231)

Organ	Cd mg/Kg	Cd Total mg	Al mg/Kg	Al Total mg	T mg/Kg	T Total mg	Pb mg/Kg	Pb Total mg	B mg/Kg	B Total mg
Adrenal	1 4	0 02	4 41	0 07	0 52	0 01	1 04	0 02	(0 39)	0 008
Aorta	2 09		9 03		0 75		1 05		(0 19)	
Bladder	(0 93)	0 19	2 33	0 5	0 21	0 04	0 39	0 08	(0 26)	0 05
Brain	0		3 55	7 1	(0 07)	0 14	0 31	0 62	0	
Heart	(1 74)	0 52	2 51	0 75	0 13	0 03	0 98	0 9	—	
Stomach & Intestine	1 20	1 40	3 25	6 50	0 44	0 85	0 48	1 16	(0 26)	
Kidney	33 1	9 93	1 80	0 54	0 14	0 04	1 27	0 38	—	
Liver	3 60	5 40	2 55	3 83	0 26	0 39	2 04	3 06	—	
Lung	(1 73)	1 [illegible]	30 8	11 6	7 46	5 16	1 21	0 85	—	
Muscle	([illegible] 8)	49 8	3 01	54 2	0 48	13 4	0 71	19 9	—	
Pancreas	2 10	0 21	2 07	0 27	0 15	0 02	1 32	0 13	(0 41)	0 04
Prostate	(1 55)	0 03	5 53	0 28	1 51	0 03	0 29	0 01	—	
Spleen	(1 96)	0 39	3 34	0 67	0 51	0 06	1 33	0 27	(1 20)	0 24
Testis	(1 31)	0 01	3 87	0 08	0 21	0 004	0 67	0 01	—	
Thyroid	2 08	0 06	5 12	0 15	0 5	0 01	0 50	0 02	0	
Skin, Skeleton, Adipose Tissue, Other Tissue	3 88	46 6	3 35	64 2	0 89	10 68	0 90	10 8	(0 18)	2 16
Sub Total known (mg)		70 24		96 56		20 46		26 80		0 338
Total with estimated skin and other soft tissue at mean value × 70 Kg (mg)		116 84		160 76		31 14		37 60		2 498
Specimens without metal	76		3		11		0		159	

Figures in parentheses indicate element missing in two or more samples

* Half or more of specimens [illegible]ly

ous junctions These signs are more like those of pyridoxine deficiency It is doubtful that zinc deficiency ordinarily can be produced in man except on very low intakes or during excessive proteinuria, during which loss of protein bound zinc can occur Some of the symptoms of beri beri have been thought to be manifestations of zinc deficiency caused by low intakes The patient shown in Table XXX exhibited acute pyridoxine deficiency twice when EDTA was given intravenously (181) probably his excessive urinary loss of protein bound zinc added to the zincuretic effect of EDTA resulted in deficiency of the zinc chelated to some pyridoxal enzymes (Fig 20 p 231)

"ABNORMAL TRACE METALS IN MAN

In Table XXXV are the concentrations and contents of the five presumably abnormal' trace metals showing some organ specificity We observe the following Aluminum was not found in three hearts but was in every other organ examined almost always in concentrations of over 1 mg /Kg and in all but six in over 3 mg /Kg It was selectively concentrated in lung (perhaps by inhalation) with a third as much in aorta and a sixth or less in prostate stomach thyroid and adrenal lowest values were in kidney Amounts were higher than those of any essential metal save zinc Cadmium appeared in kidneys in very high concentrations, with liver, pancreas thyroid containing a tenth as much Titanium appeared in lung (perhaps by inhalation) with prostate a poor second and a relatively even distribution in the other organs Lead appeared highest in liver, pancreas, spleen kidney, adrenal and aorta lowest in stomach brain and bladder The less frequently found boron appeared to be concentrated in some spleens On the other hand little evidence of concentration in any one organ was found in the cases of the

ubiquitous elements tin nickel, chromium and silver (Table XXXVI)

The obvious conclusions are that relatively large amounts of cadmium are in American kidneys and aluminum and titanium in lungs while other metals are more or less evenly distributed Furthermore there is weight for weight more of several abnormal metals in most organs than normal ones of high biological activity such as manganese copper and molybdenum On the basis of mass alone these three tables show the following when the metals are arranged according to the periodic table Silver is present in amounts equal to 2 2 per cent of copper cadmium equal to 12 per cent of zinc (50 per cent in the kidney) chromium equal to 61 per cent of molybdenum (and is more prevalent) nickel equal to 140 per cent of cobalt while there is more titanium tin and lead than manganese molybdenum and cobalt and there is more aluminum than copper Thus the order in decreasing amounts is *Zn* Al *Cu* Cd Pb Ti Sn *Mo*, Ni *Co*, Cr *Mn* Ag B according to the present estimate (essential ones in italics) Traces of gallium were found in most lungs of bismuth in 21 samples of gold in 72 samples and of thallium in 6 samples

In Tipton s second series of 24 autopsies from another (western) part of the United States (259) the findings were quite similar for the essential metals although there were somewhat less copper and zinc in most organs and molybdenum was largely confined to liver and kidney Of those now considered abnormal cadmium appeared in liver and kidney in the same concentrations as in her first series but a large majority of other organs were lacking in this element There was much less aluminum in all organs but lung (23 mg /Kg) and titanium was found in only a few bodies except for lung where its concentration

TABLE XXXVI

CONCENTRATIONS AND CONTENTS OF "ABNORMAL" TRACE METALS WITHOUT ORGAN SPECIFICITY (WET WEIGHT) (231)

Organ	Cr mg/Kg	Cr Total mg	Ni mg/Kg	Ni Total mg	Sn mg/Kg	Sn Total mg	Ag mg/Kg	Ag Total mg
Adrenal	0.28	0.006	0.26	0.005	0.34	0.007	0.05	0.001
Aorta	0.01		0.20		0.42		0.07	
Bladder	0.42	0.08	0.23	0.06	0.19	0.04	0.07	0.01
Brain	0.36	0.76	(0.14)	0.29	(0.55)	1.16	0.04	0.08
Heart	(0.13)	0.04	(0.23)	0.07	0.36	0.11	(0.26)	0.08
Stomach & Intestine	0.18	0.36	(0.39)	0.78	0.48	0.96	(0.08)	0.16
Kidney	0.08	0.03	(0.22)	0.07	0.48	0.14	0.06	0.02
Liver	0.27	0.40	(0.32)	0.48	0.65	0.98	0.07	0.10
Lung	0.72	0.50	0.47	0.33	0.95	0.66	(0.15)	0.10
Muscle	(0.18)	5.04	(0.40)	11.2	(0.51)	14.3	(0.06)	1.68
Pancreas	0.20	0.02	(0.32)	0.03	(0.53)	0.05	(0.09)	0.008
Prostate	0.28	0.01	(0.48)	0.02	0.74	0.04	0.04	0.002
Spleen	0.63	0.13	(0.72)	0.14	(0.41)	0.08	(0.23)	0.05
Testis	0.39	0.008	(0.67)	0.01	0.22	0.004	(0.08)	0.002
Thyroid	0.42	0.01	(0.25)	0.008	1.0	0.03	0.03	0.0009
Skin, Skeleton, Adipose Tissue, Other Tissue	0.32		0.37	4.44	0.53	6.36	0.085	1.02
Sub Total known (mg)		7.394		11.738		18.561		2.294
Total with estimated skin and other soft tissue at mean value×12 Kg (mg)		11.234		16.178		24.921		3.314
Specimens lacking metal	8		62		24		33	

Figures in parentheses indicate element mi[illegible]ng in two o[illegible] more sample

(266) There were 4 kidneys and livers 3 lungs 2 spleens and 1 aorta heart intestine and muscle In contradistinction to adult tissues kidneys and livers and lungs contained no titanium cadmium or tin Aluminum was not concentrated in lung although it occurred in amounts of 0 3 1 0 mg /kg in most tissues less than that of adults Titanium was found in only one body in intestine kidney and muscle Nickel occurred in one other kidney and liver tin was in both spleens and the sample of muscle and aorta On the other hand boron was in all tissues but one kidney and liver chromium and silver were ubiquitous in adult concentrations as was lead but in smaller amounts than in adults Organ specificity as determined by concentration was not found consistently *

In the bodies of 3 older infants and children (7 weeks 10 months and 2 years) there was no titanium Nickel in traces was in 1 kidney tin was found in all tissues as was lead while silver occurred in 7 of 12 specimens Cadmium was present in the 10 month-old kidney (0 65 mg /kg) and in the 2 year-old (2 75 mg /kg) but not in the 7 week *old one* The essential metals were found in distributions and concentrations similar to those of adults Manganese was if anything more concentrated zinc less so while copper was comparable showing an affinity for liver Molybdenum was in all livers while cobalt was present in only two

Within the limits of these observations, and in view of what is known the following further *conclusions* can be drawn 1 Titanium nickel and cadmium are not essential to infant life but accumulate with age 2 Aluminum chromium silver and lead either *qualify* as essential trace metals or pass through the placental *membrane* Obviously titanium nickel *cadmium and tin* do not so pass

* Analyses of 20 stillborn infants gave essentially similar results

was lower (2 8 mg /kg) Likewise lead was somewhat less frequent, being in all livers, kidneys, lungs pancreases bones, aortas and adrenals but missing in a few to many samples of other tissues Chromium and silver were also absent in one or more samples of each tissue and were found in lower concentrations, while nickel was quite uncommon Tin on the other hand, was widespread through most samples There was less gallium, the same amount of bismuth and much less gold

New elements added to the analysis were barium found in all samples of lung bone adrenal aorta, gastrointestinal tract and thyroid and in most samples of the other organs cesium in half the lungs (3 1 mg /kg) iron in all tissues in high concentrations especially spleen (280 mg /kg) lung (210 mg /kg) and liver (170 mg /kg) lanthanum in a third of spleens strontium in every tissue (0 04 0 20 mg /kg) with much (20 mg /kg) in bone and vanadium in two thirds of the lungs (0 27 mg /kg) No antimony arsenic beryllium niobium, ruthenium, thallium or zirconium were found

One can draw some tentative conclusions on Tipton s two series the first obtained on autopsies from New York Memphis and Chicago, the second from a Western city 1 Minor regional differences in exposure or accumulation of some abnormal trace metals appear, especially as regards aluminum titanium chromium silver, nickel and possibly lead 2 Molybdenum is essential only for liver and kidney 3 Chromium and nickel are probably not essential elements These conclusions apply only to the concentrations detectable by the method

Trace Metals in Infant Tissues We can gain some idea as to which metals are essential for life and which are not by examining the tissues of babies Analyses of 2 St Louis stillbirths and 2 babies living 12 hours were revealing

Some of these metals accumulate with age in Americans others do not In Table XXII Chapter V are shown examples Obviously cadmium titanium nickel and tin in all tissues and aluminum in lung increase from little or none in infants to relatively higher concentrations at older ages The striking examples are in cadmium and titanium

Trace Metals in Tissues from Uncivilized People In order to ascertain more definitely what trace metals are essential and what are not a small number of tissues from African natives in little contact with Western Civilization were obtained by Dr Perry from Uganda and analyzed by Dr Tipton (266) The ages ranged from 18 months to over 50 years there were three under 10 and four over 40 None showed any evidence of atherosclerosis in the aorta or elsewhere even fatty streaks were not seen The causes of death were several 6 patients died of acute infections No age trends in essential metals were apparent as is the case with Americans The interesting findings lay in the absence of those which might be guessed to be products of Western Civilization cadmium (in only one kidney) nickel and tin and the much smaller amounts of silver lead chromium and possibly titanium (Table XXXVII)

Conclusions Therefore it becomes apparent that nickel chromium cadmium lead silver and tin are not essential elements but results of civilization a conclusion which could be drawn from the analyses of *children*s tissues only for cadmium nickel and titanium The possibility of these six elements found in American tissues being toxic must therefore be considered Common sense excludes silver because of its low concentrations In addition we cannot rule out the possible essential nature of aluminum barium strontium and for lung of vanadium and ti

TABLE XXXVII

MEAN CONCENTRATIONS OF ESSENTIAL AND NONESSENTIAL TRACE METALS IN AMERICAN AND AFRICAN TISSUES
(ANALYSES PERFORMED BY TIPTON *et al*)
(mg /Kg Wet Weight*)

Metal	Kidney Africa	Kidney U S A		Liver Africa	Liver U S A		Lung Africa	Lung U S A		Spleen Africa	Spleen U S A		American Children 0–2 Years Kidney	Liver	Lung	Spleen
		1	2		1	2		1	2		1	2				
No cases	10	24	24	3	24	24	4	23	24	4	24	22	7	7	4	5
Mn	0 39	0 43	0 56	0 57	1 13	0 76	0 49	0 58	0 19	0 27	0 52	0 13	0 55	0 98	0 45	0 400
Cu	0 26	2 76	2 9	4 47	10 6	5 7	2 8	4 49	1 4	1 7	8 67	1 3	2 89	9 0	2 9	1 9
Zn	24 5	67 2	48	65 3	68 4	48	39 5	18 3	17	33 8	22 3	21	18 4	63 0	15 2	15 9
Mo	0	0 63	0 33	0 55(1)	1 62	1 1	0	0 18(15)	0	0	0 17(17)	0	0 85(3)	0 86	0	0
Al	5 95	1 80	0 67	5 77	2 55	0 7	28 8	30 8	23	4 88	3 34	2 2	2 1	0 64	0 69	0 52
Ti	0 64(1)	0 14	0 67(5)	1 6(2)	0 26(21)	0 5(2)	3 45	7 65	2 8	0	0 31	1 0(5)	0 47(1)	0	0	0
Cr	0 037	0 09	0 04(4)	0 0	0 27	0 03(11)	0 04	0 72	0 14	0 009	0 63(23)	0 05(13)	0 30	0 22	0 97	0 31
Ni	0	0 22	0	0	0 32(13)	0 26(2)	0	0 47	0 33(8)	0	0 72(14)	0	0 17(2)	0 13(1)	0	0
Pb	Tc(4)	1 27	0 94	0 24	2 04	1 5	Tc	1 21	0 63	0 25(1)	1 33	0 38	0 17(6)	0 51	0 09	0 37
Sn	0	0 48	0 27	0	0 65	0 56	0	0 95	1 1	0	0 41(22)	0 27(19)	1 32(3)‡	1 6(3)‡	0 13(1)‡	0 33
Ag	Tc(6)	0 06	0 02	0 015	0 07	0 03	Tc	0 15(19)	0 02(19)	0 02(3)	0 23(15)	0 06(14)	0 06(5)	0 15(6)	0 03(3)	0 12
Bi	6 3(6)†	0 49(6)	1 1(6)	1 25(1)	0 61(3)	0 9(3)	0	0	0	0 25(1)	0	0 36(2)				
Cd	±2(1)	33 1	31 0	0	3 60	3 3	0	1 73(20)	1 1(11)	0	1 96(6)	1 3(1)	1 70(2)‡	0	0	0

Differences cons dered significant from American adults a e italicized.
Numbers in parentheses ndicate specimens with metal f all specimens did not show t.
Ages of the 12 African patients 3 <10 years 2 20–30 years 2 30–40 yea s 5 40–50+ years
The African tissues were preserved n formal The per cent ash was 3 to 5 t mes greater than in f esh tissues (dehydration?) ther fore increasi g the sensitivity of the method.
† Two patients had 18 and 19 mg /Kg Bi in kidney pr bably the result f treatment
‡ In older children nly not in babies.
1 = Tipton s first series (231) 2 = second series (259)

tanium We can add to our list of possibly toxic metals bismuth and cesium

Are these extraneous elements biologically active or inert? Could one or more of these abnormal elements displace an essential metal and thus lead to metalloenzyme inhibition or combine with sulfhydryl enzymes and so inactivate them (Table XXVIII)? If so the possibility of inhibition causing dysfunction leading to disease is considerable

METALS OF POSSIBLE BIOLOGICAL SIGNIFICANCE IN THE FIRST TRANSITIONAL GROUP

It is interesting that the transitional and nearby metals in the periodic table are those with most biological activity an expression perhaps of their structures (267) Other than the known essential trace metals of the first transitional group four might serve in metalloenzymes but have at present no known function i e titanium vanadium chromium and nickel Vanadium is found in all of the animal phyla is concentrated from sea water by tunicates as an essential oxygen carrier (268) is required by aspergillus niger (269) is concentrated in certain mushrooms (270) was probably an oxygen carrier in petroleum forming animals and appears in concentrations of about 1 0 mg /kg in all plants dry weight (271) The case for it having a function in mammals is good While chromium stimulates plant growth is present in all vegetables in concentrations of 10 to 1000 γ/kg dry weight and is in many human tissues evidence for its essential nature is doubtful Nickel is probably not required by mammals although plants contain traces The role of titanium is not known although it is found in almost all human adult lungs Those concentrated in certain organs may be of more significance in metabolism or in causing diseases

TABLE XXXVIII

Effect of Transitional Metal Ions on Hepatic Metabolism in the Rat*

	Ti‡	*V*	*Cr*†	*Mn*	*Fe***	*Co*	*Ni*	*Cu*	*Zn*	*Cd*
Atomic Number	22	23	24	25	26	27	28	29	30	48
Oxidation of phospholipid fatty acid	0	+	0	—	0	−50%	0			
Dehydrogenation of PFA		+								
Oxidation of double bond in PFA		+								
Oxidation of cysteine to its sulfonic acid	—	0	sl	—		—	0			
Oxidation of thioglycolic acid	—	0	sl	—		—	0			
Synthesis of cholesterol from acetate	0	−90%	150%	125%	−60%	−40%	0	0	0	0
Synthesis of fatty acids from acetate	0	−40%	150%	55%	−40%	0	0	0	0	0
Human liver content American mg/Kg	0 26	0-Tr	0 27	1 13	1 0	(0 8)	0 32	10 6	68 4	3 6
Same African	(1 6)	0	0 02	0 57		0	0	4 47	65 3	0
Log *K* EDTA			13[1]	13 5	14 2[2]	16 1	18 5	18 4	16 6	16 5
Ratio hydralazine binding Cu = 1 0		0 3	0[1]	0 3	0 1[2]	0	0 2	1 0	0	0

Note Metal binding agents CN pyrophosphate p-aminophenol and aminopyrine inhibited the liver enzyme of Bernheim 8 hydroxyquinoline depressed hepatic synthesis of cholesterol and fatty acids while EDTA enhanced it (375)

PFA = phospholipid fatty acid

* From Bernheim and Bernheim (272 274 275) and Curran (248)

† As dichromate or chromium potassium sulfate.

** As ferric.

‡ As pertitanate

[1] As chromous

[2] As ferrous

turated fatty acids were oxidized very slightly Further more they showed that vanadium exhibited two effects dehydrogenation to produce unsaturated fatty acids and oxidation of the double bond (Table XXXVIII) The reaction was inhibited by the metal binding agents cyanide pyrophosphate fluoride p-aminophenol and aminopyrine Brain contained none of the enzyme kidney little When other metals were tested manganese and to a less extent cobalt were found to inhibit the system while nickel iron titanium and chromium had no effect (275) Manganese cobalt and titanium inhibited the oxidation of cysteine to its sulfonic acid Titanium in the form of sodium per titanate also inhibited hepatic oxidation of thioglycolic acid and ethyl mercaptan but glutathione was not oxi dized by this system (272)

Evidence for Vanadium Being an Essential Trace Metal What evidence there is for vanadium being essential to mammalian metabolism is indirect but good Aside from its use by ascidia which use it for oxidation reduction reactions at a time when they are buried in mud petroleum contains varying amounts of vanadium in a porphyrin form which has led to the hypothesis that animal organ isms and not plants originated the formation of petro-leum It is found in mammalian tissues at a fairly good concentration said to be 0 13 per cent dry weight 1 2 mg /kg for invertebrates and 0 1 mg /kg dry tissue for vertebrates (268) While its occurrence may be a chance contamination universal presence and three valence states (V^{+++} V^{++++} V^{+++++}) with an ability to release energy similar to phosphorus make it likely that vanadium may be essen tial Tipton found it only in the lung

Vanadium is a powerful stimulant to monamine oxidase (Table XXIII) Only cobalt of the others tested exhibited this property to much less degree This phenomenon

than those widespread in smaller concentrations (chromium, tin, nickel) We can supply our six rules of thumb to each

Titanium There is no evidence that titanium is necessary for normal function It can, however, act as an antimetabolite Titanium inhibits the oxidation of cysteine to its sulfonic acid (as well as thioglycolic acid) (272) but does not affect cholesterol and lipid synthesis in the rat (248) The antimetabolic action of this substance could but does not necessarily implicate it in chronic disease If involved, its only known effect is upon sulfur metabolism

When a patient with moderate hypertension was treated with hydralazine in increasing doses at four hour intervals up to 600 mg per day the only change in the urinary excretion of 11 trace metals was in titanium Taking the highest value of 4 control days this value was exceeded on 7 of 9 days of treatment on 5 it was twice as much or more on 2 6 times and on 1, 50 times greater No other trace metal so moved the control value for vanadium and chromium were exceeded three times, of lead twice and of manganese once of nine days Late results of hydralazine therapy on pairs of urine specimens before and after control of hypertension showed no essential changes in mean titanium excretion The values doubled twice and fell markedly once There were no significant changes in the urines of 5 patients with disseminated lupus (273)

Vanadium Bernheim and Bernheim have studied the oxidation of certain rat and guinea pig tissues as influenced by vanadium acetate or sodium metavanadate (274) They found that phospholipids were oxidized by the insoluble portion of liver proteins in the presence of vanadium brain and liver phospholipids and soy bean lecithin were good substrates for the vanadium enzyme system The oxidation of many other substances were unaffected Sa

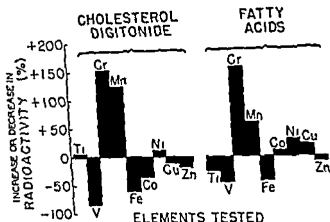


FIG 18 The effect of certain transition metal salts on the incorporation of C^{14}-carboxyl labeled acetate into cholesterol and fatty acids by surviving rat liver (From Curran G L *J Biol Chem* 210 765 1954)

adults and being ubiquitous in plants and animals (256) no role for chromium is known It markedly stimulates the hepatic synthesis of cholesterol and fatty acid in rats No other transition element save manganese had this property (Fig 18) (248) It did not affect decarboxylase and monamine oxidase

Normal urine contained less than 0 05 to 1 03 γ/L (mean <0 46) hypertensive urine less than 0 05 to 4 4 γ/L (mean 0 88) Treatment did not change the means significantly (0 67 to 0 71 γ/L) nor did hydralazine or EDTA cause a consistent loss

Chromium in liver could stimulate cholesterol and fatty acid synthesis in man There is as much however in infants as in adults Only by analyzing many more tissues from primitive areas can one determine whether or not chromium can qualify as an essential metal or if not play a part in chronic disease

strongly suggests, but does not prove, that vanadium is an essential metallic component of monomine oxidase Not until the enzyme is obtained pure and the metal identified can we say for certain that it is essential for amine oxidation

The urinary output of vanadium in human beings is fairly constant, less than 0 63γ/L (range less than 0 5 to 2 15) In hypertension, however it was increased to three times normal values, 1 95 γ/L (range 0 4 to 14 5) Treatment appears to bring back the values toward normal (2 86 to 0 93 γ/L.) However, only five of our values were initially greater than the highest normal

The pharmacological effects of vanadium have been extensively studied by Jackson (276) It is an unique element in that it causes vasoconstriction in rabbits The older literature contains equivocal reports of its effects in syphilis tuberculosis and skin diseases oral doses are well tolerated In hypertensive states, giving sodium metavanadate by mouth sometimes caused transient reduction of blood pressure but also produced chills and fever In rats it markedly depressed fatty acid and cholesterol synthesis by liver (248) (Fig 18)

It is possible therefore that vanadium deficiency either through lowered intake or exogenous abnormal metallic competitions is one of the causes of the conversion of intermittent vasospasm into sustained vasospasm Consistent with this theory are 1 Stimulation of renal monamine oxidase only by V^{+++} and V^{++++} an enzyme which destroys norepinephrine and most other circulating vasoactive primary amines 2 Reduction of pervanadate by hydralazine which also enhances monamine oxidase activity (277) Unfortunately vanadium was acutely pressor for hypertensive rats but may be depressor in dogs

Chromium Although found in all lungs of infants and

rier while its absence in many adults suggests that it is not necessary after full growth

Aluminum is almost absent from plant tissues although it represents seven to eight per cent of the earth s crust (256) Animal tissues with the exceptions of lungs and liver are said to contain less than plants (0 7 to 1.5 mg /kg in the dog and 0 5 to 3 3 mg /kg in the rat) (278 279) although a former study showed considerable amounts in human beings (brain 2 5 heart 2 1 liver 0 8 kidney 1 0 and spleen 0 7 mg /kg) but less than were found by Tipton *et al* Salts have been put into baking powders can be absorbed from cooking vessels and canning processes A considerable amount of investigation 25 to 50 years ago indicated the lack of toxicity of aluminum which is fed in antacid preparations to patients The large amount found in lungs probably comes from inhaled aluminum silicate from dust Having only one valence state in spite of its prevalence in the earth it has not been shown to have any essential role in human metabolism although by our criteria it cannot be excluded as an essential trace metal

Scandium and gallium are found in soils. The traces of gallium in human lungs probably have been inhaled since gallium is present in all aluminum minerals Both are relatively unreactive compounds with only one valence state

Comment If one were forced to choose a single trace metal to undo the harmful effects of degenerative cardiovascular diseases vanadium would be that choice for the following reasons

I Vanadium stimulates monamine oxidase thereby probably increasing the destruction of pressor vasoactive and cerebroactive amines and affecting hypertension thereby (Table XXIII)

Nickel While found in all plants and animals tissues in traces, it has no known function (256) It is almost certain that this element is not essential for mammalian growth and development There is frequent exposure in processed and manufactured foods especially hardened vegetable oils (Chapter VII) where it is used as a catalyst (containing 0 01 2 mg /Kg), and from corrosion of nickel vessels No increase was noticed in human tissues after the second decade until the seventh Therefore, although present in small amounts we cannot state whether or not it is doing harm, although it probably has little direct action It did not affect the enzyme systems of interest to this discussion and is usually inert in others

Normal concentrations in urine averaged 2 78 γ/L with a range of less than 0 05 to 12 γ/L Hypertensive urine contained twice as much, 5 53 γ/L with a range of less than 0 1 to 40 only a third however, contained more than the normal mean amount Hydralazine caused no essential changes, treatment did not alter the mean values significantly (3 99 to 5 78 γ/L) EDTA caused no apparent changes Nickel cannot at the present time be implicated in cardiovascular diseases Excessive exposure causes dermatitis or eczema

Elements of the Third Group There is no evidence that any of the elements in the third periodic group are essential for mammalian life Boron, however, appears to be essential for plant life occurring in most forms and is necessary for reproduction (256) Only traces are found in dairy products and flesh foods, since this element is fairly rapidly excreted In man however in large amounts it causes weight loss albuminuria and gastrointestinal disturbances Its presence in most tissues of stillborn infants means either that it is essential or that it is a contaminant in the mother easily transported across the placental bar

18 5) After treatment the mean value diminished from 5 47 to 2 64 γ/L. five of eight values declining Hydrala zine decreased the output to about a fourth as did EDTA The possible role of tin is undetermined although its source lies in the products of Western Civilization i e tin plate traces dissolving in some tinned foods

Silver Like tin silver is widespread occurring in every adult kidney and brain and in almost every other sample examined (225 of 258 specimens) (231) There was no tendency for accumulation with age and almost every young tissue contained it in adult concentrations (266) It was in all urine (0 8 γ/L. range 0 23 1 4) hypertensive urine contained little more than did normal (1 4 γ/L. range 0 3-4 6) Exposure to silver is constant in our society there is little or none in plants Because of the small amounts found it is doubtful that enzymatic inhibition (on copper enzymes) if present is extensive enough to cause metabolic disorders *

Lead There is lead in all human tissues at birth and during life This toxic metal accumulates especially in bone and liver As far as is known it is not an essential constituent of any living organism getting into food mainly from the use of its compounds on plants and from vessels in which food is manufactured transported or stored Shell fish may absorb lead from sea water contaminated with drainage from factories and animals from sprayed plants African tissues had little

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A knowledge of the coordination number of a metal the shape of its chelate and its periodic group allows a reasonably accurate if indirect method of predicting displacement of a known essential metal and in activation of its enzyme Conversely by inactivation studies unknown metals on metalloenzymes may be predicted (Table XXVI) cadmium and mercury displacing zinc gold silver nickel displacing copper tungsten displacing molybdenum More basic chemical knowledge will lead to more accurate predictions.

2 Vanadium unsaturates fatty acids on phospholipids and oxidizes the double bond (274)

3 Vanadium depresses the synthesis of cholesterol and fatty acids (248)

4 Vanadium lessens the formation of atheromata in experimental animals (280)

If one were to choose a single abnormal trace metal as a contributing cause of hypertension, cadmium would be the choice for reasons discussed in Chapter IV

If one were to choose three abnormal trace metals as contributing causes of atherosclerosis, chromium cadmium and lead would be the choices Tin could not be excluded

METALS WITH POSSIBLE HARMFUL EFFECTS

With some certainty one can list the metals present in American human tissues which are contaminants and which may be potentially harmful, i e silver cadmium tin, antimony arsenic gold and lead Of these, known toxicities are found in the cases of cadmium arsenic and lead large amounts of the others are less harmful but will produce diseases or disorders Just as in the cases of transitional metals those which are evenly distributed throughout all tissues (tin silver) are under less suspicion for causing disease than are those concentrated in specific organs with high metabolic activities (cadmium lead)

Tin There is no evidence that the metal is essential for life (256) It is not essential for plants Little is known of its action as an anti enzyme It was present in almost all human adult American tissues and in the spleens of infants, older children showed quantities comparable to those of adults The mean normal urinary concentration was 3 22 γ/L (range less than 0 5 to 10 0) the mean hypertensive concentration was 8 93 γ/L (range 0 9 to

cause pulmonary fibrosis (from inhalation) (282 285) renal damage (286) this toxic metal is under considerable suspicion as a cause of chronic disease Of all the exogenous abnormal metals found in adult American tissues cadmium appears the most toxic It is obviously cumulative Supposedly 40 mg by inhalation can cause death in man which is strange since the total content of adult bodies is about 120 mg and the lethal dose in rabbits by injection is 3 mg /kg (LD_{50}) (84) Hepatic and renal lesions are prominent features of acute poisoning in rabbits and rats cardiac hypertrophy is universal (286) Proteinuria and renal lesions occur in exposed workers (287) There must be obvious differences between acute and chronic effects in man especially when this metal accumulates for a life time The protein in the urine is not albumin since it appears on the heat test but is not precipitated by Esbach s reagent (287) Cadmium causes aminoaciduria in man (288) *

The source of the cadmium may lie in zinc for it is a constant contaminant of zinc ores There is probably one per cent or more in the galvanizing grade (Prime Western) as indicated by the following specifications adopted in 1911 by the American Society for testing materials last

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Cadmium causes increased excretion of the following amino acids in man exposed industrially glycine alanine glutamine tyrosine lysine histidine methyl histidine All of these except possibly lysine can act as donors of ammonia Furthermore serine excretion was increased 9.5 and threonine 33 times The amount of cadmium in the urine was in the same range 12 335 µg /L (average 20 µg /L) as we have found for hypertensive individuals (308) Cadmium was unique among four heavy metals (U Pb Cd Hg) studied by Clarkson and Kench (288) who believed that it specifically inhibited the renal tubular *reabsorption* of these amino acids. An alternative and to us preferable explanation lies in the specific inhibition of decarboxylases by cadmium thus preventing the first step of renal amino acid metabolism

There is no toxic metal which has received such concerted attention as lead (84) It has been proposed, and discounted, as a cause of many chronic diseases, including hypertension and atherosclerosis The predilection of American lead for adrenal, aorta, bone kidney, liver, lung pancreas, prostate and spleen, but not for brain bladder, muscle and intestine, suggests that it might exert an anti metabolic function The much smaller amounts in infantile and African tissues with a tendency to accumulate with age places it under suspicion There are however, no good ideas as to its effects on enzyme systems in ordinary concentrations, although lead poisoning affects the nervous system and blood It is well known that exposure to lead in industry produces definite slow accumulation Tetra ethyl lead in gasoline is a fair source (84)

While we cannot implicate this toxic, accumulating metal in any chronic disease in our society we cannot discount it as a possible etiological factor Workers in lead industries, however either develop clinical manifestation or suffer from no noteworthy disorder The earliest symptoms of sub-clinical lead intoxication are those referable to emotional stability being irritability moodiness, restlessness, excitability, common complaints in this period of history Lead salts are vasoconstrictor in perfused dogs legs and cause vascular smooth muscle to contract (281)

Cadmium The high renal concentrations of cadmium in American adults its absence in infants and in African native tissues focuses suspicion on this metal as an etiological agent in chronic disease Since cadmium can displace zinc on mercaptalbumin (243) is a highly potent inhibitor of sulfhydryl enzymes (of which Coenzyme A is an example) and at least one vitamin B_6 enzyme, can

are no good examples) we could expect on the basis of mass alone the following enzymatic inhibitions Renal 55 per cent hepatic 6 per cent pancreas 5 per cent thyroid 5 per cent adrenal 8 per cent aorta 5 per cent intestine 5 per cent brain 0 per cent Obviously a 5 per cent reduction of metabolic activity would probably be unmeasurable while a 50 per cent would manifest itself in disorder which could lead to disease

Renal decarboxylase is inhibited *in vitro* at a lower concentration of cadmium than that present in adult kidney Other enzymes known to be inhibited *in vitro* are leucine aminopeptidase carnosinase succinic dehydrogenase choline oxidase (Table XXXVIII) possibly through sulfhydryl binding If other metalloenzymes are specifically inhibited such as vitamin B_6 enzymes it is obvious that effects of low concentrations could be profound and in the case of vitamin B_6 result in a conditioned local deficiency

As in the case of zinc and lead cadmium can be dissolved in slightly acidic media Therefore foods and waters coming in contact with cadmium could become contaminated by traces There are three possible sources 1) Water is usually piped in American houses through galvanized zinc coated iron pipes If soft aerated in muncipal water stations to contain carbon dioxide and chlorinated appreciable quantities of zinc lead and presumably cadmium could be dissolved from the galvanized coat If hard however insoluble carbonates are laid down on the coating protecting it from solution and corrosion Water softeners probably would not soften water enough to corrode zinc Chlorinated water even when hard takes up zinc.* 2) Carbonated beverages are acidic and will take up zinc lead and presumably cadmium from galvanized or zinc lined

* A probable source of abnormal trace m tals in some soft and acid water areas is in the corrosion of hot water heaters

revised in 1949 The maximum impurities allowable are (per cent)

	Lead	Iron	Cadmium	Total Not Over
Special High Grade*	0 006	0 005	0 004	0 010
High Grade*	0 07	0 02	0 07	0 10
Intermediate	0.20	0 03	0 50	0.50
Brass Special*	0 60	0 03	0.50	1 00
Selected*	0 80	0 04	0 75	1.25
Prime Western	1 60	0 08	—	—

* It shall be free from aluminum

Since Prime Western zinc is the grade used largely in galvanizing and no limits for cadmium are provided, it is therefore probable that zinc coating so widely used in pipes and food processing and brass are likely sources of the cadmium found to accumulate in human tissues

Cadmium poisoning has been reported in human beings drinking acid beverages made in cadmium plated (yellow tinged) ice trays (289)

The high concentrations in adult human kidneys (33 1 mg /Kg or about 10 mg of the metal) with secondary affinities for liver pancreas and thyroid and lesser amounts (±1 mg /Kg) in adrenal aorta stomach and intestines suggests that antimetabolic activity could be exerted especially in those organs of greatest import to essential functions Its absence in most hearts muscle tissue spleens and all brains is interesting indicating that proteins or enzymes in those areas do not chelate or bind this metal readily for cadmium is readily chelated by sulfur and nitrogen ligands (its affinity for EDTA, for example exceeds that of zinc cobalt ferrous iron and manganese) Its affinity for zinc suggests that it might interfere with zinc enzymes displacing the essential metal as it does on serum albumin (by displacement from indole groups) If zinc enzymes were inhibited by cadmium (although there

that zinc poisoning which does not occur in animals fed small amounts may be actually caused by contaminating cadmium

The source of cadmium which does not occur in plants is obviously in the products of Western Civilization *

Other Metals Analyses for other commonly occurring possibly toxic metals such as arsenic antimony and bismuth of group V A have revealed no striking accumulations in human tissues There may be as much as 0 3 parts per million of arsenic in man much of it in hair and nails derived from fish and sea foods or from contaminants of food Arsenic displaces phosphorus in essential phosphate mechanisms but the amounts are probably too small to cause functional disorders and habituation or tolerance develops Antimony can gain access to foods from enamels solders tin foil rubber and insecticides The normal amounts in human tissues are not known but presumably it also displaces phosphorus. Bismuth was found by Tipton in only a few bodies in small amounts in liver and kidneys (12 of 18 cases) and is probably not to be considered of universal import Mercury appeared in a surprisingly large number of kidneys analyzed by Griffith *et al* from patients with congestive heart failure who had supposedly never received diuretics containing this metal (260)

Cardiovascular Implications Aluminum and strontium were present in all samples of American heart muscle These metals plus lead and tin were found in all aortas In kidney there was the additional metal cadmium in liver these five and silver In adrenals were the same six and

* Preliminary single analyses of five bottled drinks revealed a carbonated water <1 a popular carbonated drink 11 a citrous drink 1 a grape juice 15 a whiskey 55 in parts per billion. Three of these values are considerably higher than those of normal urine The grape juice contained relatively much nickel tin and lead.

containers in which they are piped, prepared or bottled Lemonade made in galvanized pails has produced *acute gastroenteritis* The widespread use of carbonated acidic drinks ("pop) in this country is a possible source which has not been explored 3) Acidic foods prepared in zinc lined containers, especially vegetables, can absorb cadmium and lead

According to Monier Williams (256) zinc is taken up by the following ingested products presumably cadmium is also dissolved Chlorides in water, *chlorinated water, carbonated water*, oxygenated water (from brass) soft acid water (from brass), milk (during pasteurization) milk (from bottle caps), alcoholic, acid or saline liquids, especially *wine, vinegar* soup orangeade lemonade, beer *molasses (from zinc coated vessels in sugar refineries)*, maple sugar, honey, chocolate and candy *(wrapped in zinc foil)* gelatin (from zinc coated vessels and wire netting on which *it is dried) dried fruit (from drying trays)*, jam (from *pans) Poisoning* resembling that from cadmium has been reported from soda water, rain water (collected from galvanized roofs or stored in tanks) stewed apples (from galvanized iron vessels) root beer, cider *Coffee is floated* and dried on galvanized trays All galvanized iron vessels are suspects since electrolytic action between the iron and the zinc may be initiated by moist foods

Cadmium is widely used for washing machines electric *cooker parts and refrigerator trays It is* absorbed by weakly acidic *foods*, sugars (jam) wine *tomatoes*, fruit fruit juice ice cubes (from acidic water?) coffee cooked food gelatin and attacked by lactic succinic citric and tartaric acids Possible sources of contamination *besides* galvanized zinc and cadmium plated vessels, are *solders*, fruit insecticides The similarity of known sources of acute poisoning and *those of zinc* are remarkable and suggest

presence in large quantities and its lower affinity for some proteins than other metals with a higher EDTA metal stability constant. Of course excessive quantities of metals obey the law of mass action and can be partly removed minor quantities cannot until better chelators are de veloped for fairly specific purposes

What good will it do our patients if the e abnormal metals are removed from their bodies? We do not know The subject of metals and chronic diseases is barely begin ning to be appreciated Forbes *et al* say for example It is conceivable that the continuous ingestion of infinitesi mal amounts of these metallic elements present in natural foods leading to their very gradual accumulation in the tissues may contribute to the processes of senescence in proportion to the degree with which they are combined with tissue proteins (apoenzymes) and the extent to which they inhibit or distort enzyme action in such combinations (290) While this may be true it is more likely that many of the diseases common only to our Civilization may be caused by the nonessential metals contaminating our foods, as a result of our industrial habits We may be pleasantly surprised at the therapeutic results of their removal Scleroderma has already been completely relieved in at least one instance by EDTA (291)

A logical therapeutic regimen within the limits of pres ent-day vision is a concerted effort at removal by relatively nonspecific chelators followed by replacement of essential metals so removed Generalized deficiency states of the essential metals iron cobalt copper and molybdenum is probably unlikely under such a regimen manganese and zinc may require replacement Too much of an essential metal however probably can cause as much disorder as too little When deficiencies are recognized they can be treated

Local deficiency states caused by abnormal metal com

boron chromium gold and nickel Barium was found in all aortas Cardiovascular organs, except the heart, appear to have the ability of accumulating at least four abnormal metals Several are known antimetabolites for man or living organisms (Table XXXII)

Many of these metals are found in normal and hypertensive urine (308 310), cadmium and manganese being increased in the latter Specific enzyme inhibition has been demonstrated for several which may play a part in cardiovascular (311) and other chronic diseases (312)

CLINICAL IMPLICATIONS

At the present time, there are no known methods for removing from the human body one or more of these abnormal metals and leaving the essential ones Furthermore, the rules of chelation make such a procedure inconceivable Each metal probably has a different stability constant for different proteins and removal therefrom would require toxic amounts of very strong chelators which would complex or bind such essential metals as iron and magnesium The situation may be roughly analogous to that in argyria where silver is permanently deposited in the skin and cannot be removed by any known method

On the other hand certain metals might be eliminated from the body by the judicious use of chelators with specialized groups Thus aurintricarboxylic acid fairly selectively binds beryllium in a soluble lake reversing some of the actions of beryllium poisoning (232) A sulfur containing chelator might sequester cadmium although the affinity of this metal for renal tissue is high BAL giving up cadmium to kidney which it has displaced from other areas While such oxygen nitrogen containing chelators as EDTA and its relatives prefer copper and nickel to other elements in the first transitional group, in actual practice zinc is largely removed, probably because of its

Chapter VII

SOME MECHANISMS IN ATHEROSCLEROSIS

INTRODUCTION

Although atherosclerosis is usually a disease of Western Civilization it has been observed in nomads To begin to understand its pathogenesis one must consider the influences which civilization may contribute and those which can lead to the disease in uncivilized people A brief discussion in a monograph on hypertension is justifiable for hypertensive patients are prone to develop the disease treated hypertensive patients die mainly of its effects (319) hypertension accelerates its progress and there may be some basic factor common to both

Atherosclerosis can occur without diastolic hypertension Severe degrees of the disease in the aorta causing loss of elasticity produce systolic hypertension because the pipes are hard but do not of themselves cause elevated diastolic pressure Contrariwise hypertension can persist without significant atherosclerosis especially in China (8 313 440)

In 1941 Snapper made some pertinent comments which are lately being appreciated (8) Another point which must be specially mentioned is the infrequency of arteriosclerosis in North China The rarity of arteriosclerosis is proved by the scores of middle aged patients dying from all sorts of diseases showing hardly any sclerosis at autopsy Extensive arteriosclerosis certainly does occur in North China but the thickened inelastic aorta with the widely

petition with metalloenzymes pose a more difficult problem of replacement, especially when the abnormal metal is more firmly complexed to enzyme than is the essential one If a vitamin coenzyme is involved in the metalloprotein complex, it need be also replaced Local enzymatic deficiencies have been postulated, for example, in the case of vitamin B_6 deficiency in the skin causing seborrheic dermatitis (292)

We may speculate on the results of such a therapeutic trial aimed at restoration of normal metallic balances If the copper involved in the formation of melanin were partly displaced by an exogenous abnormal metal deposited in the skin, or were removed therefrom, the resultant grey hair and deficient tanning could be reverted to normal by removal of the offending metal and replacement of the copper If the excessive cadmium nickel lead or titanium could be removed from human tissues the part that one or more of these metals might play in hypertension atherosclerosis malignant tumors arthritis collagen diseases or allergic responses might be mitigated, none of these conditions can be excluded as not being influenced by abnormal trace metals The field is wide and the frontier untrod

Perhaps some day when these hypotheses are more firmly proven and specific metals strongly implicated in the causation of diseases industry will prevent contact of foods with those metals shown to accumulate in American tissues such as nickel cadmium tin and lead The subject would then enter the field of preventive medicine, rather than that of therapeutics where it now lies

PATHOGENETIC FACTORS

According to Friedman *et al* (314) the following schema invokes the multiple etiological factors and illustrates the pathogenesis of atherosclerosis

Time ×	Intrinsic (?) Intimal Derangement	× Quantitative and Qualitative Alteration of Plasma Lipids Including Cholesterol	× Blood Pressure	= Atherosclerosis

We will consider each of these factors separately

1 Blood Pressure Rarely does long standing diastolic hypertension in Caucasians exist beyond the age of 40 without atherosclerotic lesions being found in aorta major arteries or coronaries Experimental hypertension is necessary to induce atherosclerosis in the rat a resistant animal and is desirable in the dog Fat in serum can be made to ✓infiltrate the walls of arteries under high pressure especially if the intima is injured (315) Even in normotensive persons lesions develop at the sites of changes of pressure such as the mouths of the renal arteries in the Circle of Willis and at the bifurcation of the aorta (316 317) Atherosclerotic gangrene seldom occurs in the arm but is frequent in the leg where the hydrostatic pressure of the blood in the upright position is added to the blood pressure (318) These lesions are believed to be the result of pressure causing deposition of insoluble cholesterol or its esters subintimally either by forcing them into the vessel walls or preventing their diffusion out after entrance via the vaso vasorum

2 Intimal Injury Mechanical injury to the intima of dogs results in the formation of atheromata (320 322) It is difficult to understand how injury can occur at a normal pressure although acute hypertension in animals (323)

ulcerated intima so frequently found in autopsies in the Western part of the world, is decidely rare here This explains why the genuine angina pectoris syndrome, and also the picture of coronary thrombosis with myocardial infarction are only rarely encountered During the past two years we saw three possible cases of this disease in the combined material of the common and private wards the outpatient department, and the very busy emergency clinic of the hospital, although in every doubtful case repeated electrocardiograms with four leads are taken and scrutinized with utmost care However in December 1940 one classical example of coronary thrombosis with typical findings at the autopsy was observed The rarity of coronary thrombosis in North China is the more striking because the increase of the frequency of this affection in America and Europe is appalling Even in diabetes mellitus extensive arteriosclerosis must be infrequent in North China because diabetic gangrene is as rare here as senile gangrene

'It is difficult to give an explanation of this characteristic feature of geographic pathology One can, of course, fall back on the equanimity of the Chinese, but the differences in nutrition of Chinese and Westerners may give a better explanation Arteriosclerosis begins as a fatty infiltration of the intima of the vessel walls Quantitative and qualitative differences exist between the lipoid content of the Chinese and the foreign diets as has been mentioned before The Chinese diet contains only small amounts of cholesterol but considerable quantities of unsaturated acids especially of linoleic and linolenic acid It is certain that the average cholesterol content of the blood of the Chinese is lower than that of Westerners and this gives perhaps an indication why the tendency to lipoid infiltration of the vessel wall is so much smaller among the Chinese

metal interference (with decarboxylases for example) that in liver by marginal concentration of vitamin B_6 excessive saturated fatty acid load and possibly metals There is no evidence for a generalized deficiency state except for the high incidence of dandruff in the population believed by some to be dependent upon vitamin B_6 and fatty acid imbalance

3 Trace Metals The synthesis of cholesterol and fatty acids by surviving rat liver can be influenced by metals of the first transitional group (Fig 18) Chromium and manganese have a pronounced enhancing action vanadium a depressant one (248) Vanadium also promotes unsaturation of phospholipid fatty acid and oxidation of the double bond opposed by manganese (Table XXXVIII) There is no evidence however that chromium is implicated in the hypertensive process In American tissues hepatic chromium is much less concentrated than manganese a known lipotropic agent (231 259) (Chapter VI) Many metals directly affect oxidation of unsaturated fatty acids *in vitro* Hydrogenation to harden or saturate them is accomplished commercially mainly by copper and nickel sizeable quantities enter the fat during processing (256) Cadmium however inhibits at least one vitamin B_6 enzyme DOPA decarboxylase (Chapter V) although it does not affect hepatic synthesis of cholesterol in the rat (331) Obviously we need to know much more about the effects of abnormal metals on the enzymes concerned in fatty acid and steroid synthesis

A significant series of experiments were done by Curran and Costello in rabbits (280) Hypercholesterolemia was induced by feeding cholesterol at the 3 per cent level for 4 weeks On resumption of a normal diet cholesterol levels usually fall slowly Half the rabbits were fed vanadium as $VOSO_4$ (0 05 per cent) for 6 weeks There were

and chronic hypertension in man will result in deposition of lipid in the high pressure areas This process is hastened if lipid in blood is elevated (324)

Subintimal ' injury ' from biochemical alterations in the mucopolysaccharides of collagenous tissue results from pyridoxine deficiency in the monkey (325, 326) The changes found resemble the earliest lesions of atherosclerosis (327 330) * It is possible, therefore, that a conditioned vitamin B_6 deficiency not only affects the enzymes concerned in protein metabolism resulting in hypertension (Chapter V) but also initiates the arterial lesions in which cholesterol is deposited

It should be emphasized that the vitamin B_6 deficiency postulated in this discussion is a local, conditioned deficiency state and not a generalized one, and that it involves enzyme systems in kidney and liver but not necessarily in brain blood forming organs or skin The complex Schiff base of pyridoxal and amino acids is a strong chelating agent, for example, and could form a stable chelate with several abnormal metals *Generalized* deficiency of vitamin B_6 is accompanied by low blood pressure and skin manifestations similar to seborrheic dermatitis and ariboflavinosis A *local* deficiency in kidney could be induced by marginal concentrations of vitamin B_6 and especially trace

* Rinehart and Greenberg state Arteriosclerotic lesions develop regularly in the rhesus monkey subjected to prolonged pyridoxine deficiency The initial lesion is characterized by the accumulation of a mucinous substance in the intima and to a less extent in the media of the arteries involved This material exhibits the metachromatic staining property characteristic of mucopolysaccharides Associated with the accumulation of this substance cellular proliferation occurs and collagenous and elastic tissue fibers are formed Studies of human arteriosclerosis indicate that basically similar sequences are seen in the evolution of the human disease The morphologic features of the experimental vascular lesions and those occurring in man are similar The possible role of pyridoxine deficiency in the etiology of human arteriosclerosis remains to be determined (32)

examine the possibilities we can look at factors of suspicious import in hypertension which could conceivably influence the development of cholesterol-containing atherosclerotic plaques in arteries

There is no good evidence that the blood cholesterol is higher in American hypertensive than in normotensive people Therefore the increase in the rate of progression of atherosclerosis in hypertension is probably largely due to intravascular filtration pressure causing deposition of lipids through the intima (319)

Hypertension and atherosclerosis however may be interrelated in a more fundamental fashion than by mechanical excessive filtration of cholesterol through intima by the high pressure when there are adequate cholesterol levels in blood 1) A conditioned vitamin B_6 deficiency may involve both disorders 2) abnormal trace metals may not only affect the hypertensive process but increase cholestrol synthesis At this point the reader may wonder whether or not the author has an obsession with vitamin B_6 and its functions Upon careful thinking in terms of enzymatic mechanisms this coenzyme continually obtrudes itself into possible schemata derived from experimental and clinical data both in hypertension and in fatty acid metabolism

We must turn to epidemiologic data for evidence that there is *no* common denominator of these two diseases Some atherosclerosis but no hypertension has been found in Alaskan Esquimaux (332) the incidence may be smaller than in whites Hypertension is extremely common in Hawaiian sugar plantation workers *as is atherosclerosis* but severe coronary sclerosis is less frequent than in Caucasians (333) Atherosclerosis *is said* to be prevalent in Kirghiz nomads as is contracted kidney (from hypertension?) (334) Snapper observed hypertension but little

no significant differences in hepatic cholesterol at the end of this time, but aortic and serum cholesterol values were about half the controls in the animals receiving vanadium Likewise the livers of these rabbits incorporated C^{14} labeled acetate into cholesterol at a markedly reduced rate Thus, both endogenous synthesis and aortic deposition of cholesterol were depressed considerably by vanadium

Therefore, vanadium as a possible essential metal manganese as a known one and chromium as an abnormal one can be implicated in cholesterol metabolism As will be considered below any metal interfering with renal enzymatic mechanisms also cannot be excluded as an indirect participant in vascular damage, initiating deposition of lipid in arteries

SOME COMMON DENOMINATORS OF HYPERTENSION AND ATHEROSCLEROSIS

One anatomical common denominator between the two diseases lies in the location of the lesions When involving the renal arteries or their mouths reduced renal blood flow may result in diastolic hypertension of sufficient degree to restore flow to normal (Chapter IV) These lesions have been demonstrated frequently in hypertensive patients and confirmed by physiologic measurements It is also possible that chronic hypertension may influence deposition of lesions in the smaller renal vascular areas which of themselves cause further hypertension on a renal ischemic basis Thus will one vascular disease *worsen another* in a vicious circle

There may be chemical common denominators which predispose human beings to both hypertension and atherosclerosis This is no new idea, for many people have wondered about the relationship The geographic and racial distributions of the two are often quite similar To

is probable that local deficiency of vitamin B_6 due to the interference of its trace metal by an extraneous one could probably have the same result The intimal injury in vitamin B_6 deficient monkeys is exactly like the earliest lesions of atherosclerosis

Comment Some derangement common to both diseases may be present but atherosclerosis with which the American public is riddled is more frequent in the population than is hypertension Therefore while atherosclerosis can and does occur without hypertension the contrary is unusual in our civilization but frequent in others Both abnormal trace metals and local pyridoxal deficiency may be implicated

THE ROLE OF FAT AND OTHER LIPIDS

Let us review modern ideas on the pathogenesis of the lesions other than the factors already discussed Most of the recent interest in the subject has centered on fats Quite a case can be made for the role of cholesterol which is largely carried by lipoproteins as a strong link in the *chain of* reactions leading to the formation of plaques the subject has had its ups and downs since 1914 but probably is here to stay As Aschoff so aptly put it From plasma of low cholesterol content no deposition of lipoids will occur even *though* the mechanical conditions are favorable (327)

Normal Cholesterol Levels in Blood What is the normal level of blood cholesterol? That is a difficult question to answer The levels found in Europeans and especially Americans may not represent normal *values* but rather average values in a population subject to the disease If so we should look elsewhere at healthy adults to determine our normal standards and thus our therapeutic aims in controlling and reversing the atherogenic tendencies of

atherosclerosis in poorer Chinese (8) sometimes the condition was malignant Therefore, while the two diseases usually are found together in the same population, exceptions in both directions may exist Coronary sclerosis however, is much more common in hypertensive than in normotensive people (335) Coronary sclerosis may be a different disease than aortic atherosclerosis or may be a different manifestation influenced by local cardiac factors Likewise cerebral atherosclerosis may differ Undoubtedly however, the underlying biochemical alterations are a common denominator The epidemiological data does not prove the lack of association of the atherosclerosis seen in hypertensive states in Western countries but merely indicates that each condition can in some areas of the world, exist separately

Experimental evidence, however, which points to a close association between the kidney and atherosclerosis is accumulating Holman was the first to show that arteritis appeared in dogs fed butter only when the kidneys were damaged by uranium or mercury (336) Methods for producing experimental atherosclerosis have been developed which fit into Friedman s schema if kidney is substituted for blood pressure Renal damage will produce atherosclerosis in rats and rabbits fed cholesterol (331, 332) Nephrectomy causes an increase in plasma cholesterol and low density lipoproteins especially when protein is fed (385) Unfortunately we have been unable to cause the disease by feeding rats and chicks stearate and any one of eleven metals

One further factor which may be common to the two disorders is in pyridoxal deficiency Olsen and Martindale were able to produce chronic hypertension in young rats by desoxypyridoxine, an antimetabolite for vitamin B_6 (194) While theirs was a generalized deficiency state, it

the populations with high values Just because a disease is common does not make it or any one of its measurable parameters normal

Considerable information comes from studies in other than Western countries (Table XXXIX) If these values for blood cholesterol be correct as there is little reason to doubt the normal range is 120 to 160 mg per cent higher values may be ascribed to dietary influences or their concomitants That environment and an increasing standard of living may affect blood lipids was well shown by Toor *et al* in their study of recent immigrants to Israel compared to immigrants living 20 years or more in that country (338) (Table XL)

In Table XLI are shown some wide variations in total cholesterol and other lipids in blood done by analytic methods which are considered quite accurate from various Western countries The variations are unexplained Page *et al* tried to check the differences between their analyses done in New York (339) and Boyd s done in Ontario (340) they state our results for cholesterol determined in the presence of the other lipids are likely to be low rather than high For the fact that our normal cholesterol values range so much higher than those of Boyd (in Ontario) and of Gardner and Gainsborough (in England) we therefore lack an explanation We can find no source of error for our results and none is obvious for theirs" Since Boyd s normal subjects were taking the standard high fat diet customary in this country (340) it is possible that an en vironmental factor not present in England and Ontario but influencing levels in New York was operating

Effect of Blood Levels of Total Cholesterol Most in vestigators believe that coronary atherosclerosis does not occur to any appreciable extent when blood cholesterol is low Furthermore some reversal of atheromata is in

TABLE XXXIX

SOME BLOOD CHOLESTEROL LEVELS IN HEALTHY MALE SUBJECTS

Location	*Race*	*Mean Age*	*Mean mg %*	*Dietary Fat %*	*Method*	*Author*
Calcutta	Hindu & Mohammedan*	—	140	—	Myers & Wardell	Bose & De (1936) (378)
Calcutta	Indian	—	116	—	(Whole blood)	Boyd & Ray (1928) (379)
	Indian	—	140	—	(Whole blood)	Ghose (1933) (380)
E Arctic	Esquimo	—	141	High	(Bloor)	Corcoran & Rabinowitch (1937) (381)
Alaska	Esquimo	—	203	35	(Bloor)	Rodahl (1954) (332)
S Africa	Bechuana	21–38	149	<20	(Lieberman Burchard)	Walker & Arvidsson (1954) (382)
	Basuto	21–40	153	<20		
	Bantu	21–40	167	<20		
	Bantu Westernized	21–40	178	20–25		
	Europeans	21–30	206	30–35		
	Europeans	41–50	238	30–35		
Spain	Laborers	45	210	27	(Lieberman Burchard)	Keys *et al* (1954) (383)
	Professional	45	254	40?		
Naples		45	231	20		
London		45	252	35		
Minnesota		45	247	40		
Israel	Yemenites	45–54	160	—	(Bloor)	Toor *et al* (1954) (338)
New York		20–91	232	—	—	Page *et al* (1935) (339)

NOTE In another survey Bronte-Stewart *et al* showed that in the Cape Peninsula Africa cholesterol values done by the same method were Bantu 166 3 ± 47 2 Cape Colored 204 1 ± 54 8 and European 234 0 ± 52 9 mg per 100 ml serum (440)

* Twenty four women 76 men

TABLE XLI

VARIATIONS OF PLASMA CHOLESTEROL LEVELS IN WESTERN COUNTRIES*

Author	Location	Date	Total	Free	Phospholipids
Gardner and Gainsborough	England	1927	153 ♀ 169 ♂	54 50	
Man and Peters	Connecticut	1933	207♂		222
Boyd	Ontario	1935	177♂	52	185
Page *et al*	New York	1935	232♂	82	181
Peters and Man	Connecticut	1943	194	54	240
Gertler and Garn	New York	1950	224		299
Gubner and Ungerleider	New York	1949	211		
Keys	Minnesota	1949	218		
Kornerup	Denmark	1950	203	55	172
Block *et al*	Minnesota	1951	181		234
Perry and Schroeder (Hypertensive)	St Louis	1955	226		

NOTE The earlier determinations were done by digitonide precipitation and the later usually by acetic anhydride which tends to give lower values (439)

* From Page *et al* (339) and Katz and Stamler (356)

TABLE XL

COMPARISON OF SERUM LIPIDS CHOLESTEROL AND LIPID-PHOSPHORUS IN THE OLD AND NEW YEMENITE IMMIGRANTS IN ISRAEL*

Age Group	Total Lipids mg %		Relative Difference %	Total cholesterol mg %		Relative Difference %	Lipid phosphorus mg %		Relative Difference %
	Old Immig	New Immig		Old Immig	New Immig		Old Immig	New Immig	
Men									
35–44	639	558	11	188	146	28	9 3	8 4	10 0
45–54	695	602	15	195	160	22	9 6	8 9	7 8
55–64	654	603	8	191	158	20	10 4	9 5	9 8
Women									
35–44	688	620	11	196	172	14	9 8	9 8	0
45–54	731	607	20	213	170	22	10 6	8 7	21 0
55–64	732	615	19	220	205	7	10 9	10 3	0 3

For the purpose of this Table values of the new immigrants group were considered as 100%
New Immigrants = living in Israel 3 to 5 years Old immigrants = living in Israel over 20 years
* After Toor Agmon and Allalouf (338)

acid esters of cholesterol regardless of the length of the carbon chain melt at higher than body temperatures the lowest is for oleate (44 5°C) and linoleate (42°C) compare stearate (82 5°C) and palmitate (90°C) (348) Therefore variations in solubility and melting point may determine deposition of these esters in the lesions Solubilities of the cholesterol esters of β-lipoproteins believed to be of atherogenic importance (350) are not known

Since pyridoxal is concerned with hepatic desaturation of di and tri unsaturated fatty acids to tetra and hexa forms (349) this coenzyme could influence the type of ester Experiments in our laboratories however have failed to show that vitamin B_6 raises the iodine number of plasma lipids when given for a week or 10 days the tendency was for it to fall (Table XLII) Surprisingly enough the iodine numbers of blood lipids was found to be much higher in Chinese (8) than in American patients (Table XLIII) probably a reflection of their high unsaturated fatty acid diets

Other Lipid Substances We have not considered the phospholipids of plasma nor the chylomicrons containing neutral fat nor the lipoprotein fractions which carry cholesterol These are complicating parameters whose significance is unclear Their relations are shown in Table XLIV Keys has said At the present time (1951) it is entirely unjustified to attribute to G measurements any special virtues beyond that for simple cholesterol measurements for the prediction of atherosclerosis or the estimation of the activity of the atherosclerotic process (351) The type of lipoprotein its solubility and its physical characteristics however may have atherogenic properties These large molecules transport lipids and steroids in blood (352) Therefore an increase in β-lipoproteins may do something to the process directly or indirectly but at

ferred in patients dying of debilitating diseases (341) with depressed blood lipids In the monkey, both vitamin B_6 deficiency and elevated blood cholesterol are necessary to induce atheromata, levels are higher in deficient than in normal monkeys fed cholesterol (324) Since not only cholesterol, but other suspended particles will filter through intact intima (342, 343), the blood level is the obvious factor in determining whether or not this sterol is deposited in the lesions There is no evidence at present, however, to implicate vitamin B_6 in the synthesis of cholesterol

The deposits in the aortic plaques are mainly esterified cholesterol (316, 344 347) Schoenheimer found that there was a steady increase with age of both free and cholesterol ester extractible from the aorta as atherosclerosis developed The proportion of free cholesterol to bound cholesterol, however was relatively constant (22 5 to 31 9 per cent) showing no age trend, while cholesterol ester calculated as oleate tripled at older ages with advanced lesions In other words although the aortas contained more extractible fat, the cholesterol esters in atheromatous aortas were relatively greatly increased Most of the esters were oleate palmitate and stearate, with small amounts of unidentified unsaturated fatty acid esters There was also a marked increase in aortic phosphorus and lecithin with atheromata and a decrease in free hexosamine (433)

The Nature of Cholesterol Esters Cholesterol is a very insoluble substance how all of it is transported in blood is not known but most is carried by lipoproteins Esters of stearate palmitate and oleate are found in human blood and tissues (348) Cholesterol linoleate is considerably more soluble and linolenate much more soluble than is the stearate ester Solubility in tissues and tissue fluids may be of considerable importance Although all fatty

acid esters of cholesterol regardless of the length of the carbon chain melt at higher than body temperatures the lowest is for oleate (44 5°C) and linoleate (42°C.) compare stearate (82 5°C) and palmitate (90°C) (348) Therefore variations in solubility and melting point may determine deposition of these esters in the lesions Solubilities of the cholesterol esters of β-lipoproteins believed to be of atherogenic importance (350) are not known

Since pyridoxal is concerned with hepatic desaturation of di and tri unsaturated fatty acids to tetra and hexa forms (349) this coenzyme could influence the type of ester Experiments in our laboratories however have failed to show that vitamin B_6 raises the iodine number of plasma lipids when given for a week or 10 days the tendency was for it to fall (Table XLII) Surprisingly enough the iodine numbers of blood lipids was found to be much higher in Chinese (8) than in American patients (Table XLIII) probably a reflection of their high unsaturated fatty acid diets

Other Lipid Substances We have not considered the phospholipids of plasma nor the chylomicrons containing neutral fat nor the lipoprotein fractions which carry cholesterol These are complicating parameters whose significance is unclear Their relations are shown in Table XLIV Keys has said At the present time (1951) it is entirely unjustified to attribute to G measurements any special virtues beyond that for simple cholesterol measurements for the prediction of atherosclerosis or the estimation of the activity of the atherosclerotic process (351) The type of lipoprotein its solubility and its physical characteristics however may have atherogenic properties These large molecules transport lipids and steroids in blood (352) Therefore an increase in β-lipoproteins may do something to the process directly or indirectly but at

TABLE XLII

IODINE NUMBER OF SERUM LIPIDS BEFORE AND AFTER ORAL B_6*

Patient	Age	Sex	Iodine Number: Before Vitamin B_6	Iodine Number: After Vitamin B_6	Total Dose mg	Diagnosis
T C	77	♂	87			
G E	76	♂	100	58	250	Myocardial infarction Parkinsonism atherosclerosis
C H	61	♂	54	45	300	Myeloid leukemia aortic stenosis atherosclerosis
B H	53	♀	60	56	250	Metastatic carcinoma atherosclerosis
L J	58	♂	53	59	150	Metastatic fibrosarcoma
R LeG	19	♀	83	70	300	Myocardial infarction atherosclerosis
M R	68	♀	42	37	250	Fracture spine accident
G S	74	♂	59	69	250	Atherosclerosis diabetes mild
G B	58	♂	33	67	250	Atherosclerosis carcinoma of prostate
T B	74	♀	78	89	150	Hypertension atherosclerosis
M J	50	♂	126	77	350	Metastatic carcinoma of breast atherosclerosis
E K	64	♀	124	71	350	Pulmonary insufficiency and fibrosis atherosclerosis
F S	55	♂	71	34	350	Atherosclerosis lobar pneumonia convalescent
			62	55	300	Atherosclerosis hypertension
Mean	61		74	61		
S D			27 6	15 0		

From data of Perry Schwartz Hager and Schroeder
* Fifty mg pyridoxal HCl per day

TABLE XLIII

IODINE NUMBERS OF SERUM LIPIDS WHITE PATIENTS

Patient	*Age*	*Sex*	*Total Cholesterol mg /100 ml*	*I₂ No*	*Atherosclerosis Diagnosed*	
T K	64	♀	96	71	+	Lobar pneumonia convalescent
P M	65	♂	101	94	+	Pulmonary insufficiency bronchiectasis
F I	59	♂	106	56	+	Rheumatic heart disease
M S	54	♂	111	70	+	Myocardial infarction
L J	68	♂	119	82	+	Myocardial infarction
J D	67	♂	124	89		Prostatic hypertrophy emphysema
G D	62	♀	131	63		Hyperthyroidism
R S	68	♂	133	97	+	Metastatic carcinoma of stomach
K S	49	♂	134	61	+	Inferior vena caval obstruction
B H	67	♂	1[illegible]8	70	+	Cerebral thrombosis
M J	50	♂	182	124	+	Pulmonary fibrosis and insufficiency
T S	55	♂	191	61	+	Hypertension
G B	58	♂	206	78	+	Hypertension
I S	48	♀	210	88	+	Angina pectoris
T B	74	♀	231	126	+	Metastatic carcinoma of breast
G H	45	♂	242	73	+	Angina pectoris
J M	60	♀	550	162		Nephrotic syndrome
I T	44	♂	>500	102		Amyloidosis nephrotic syndrome
J B	65	♀		84	+	Cerebral thrombosis hypertension
M C	53	♀		76		Vascular tumor of brain
J H	54	♂		70	+	Hypertension aneurysm of Circle of Willis
A H	63	♀		62		Chronic cystitis
V V	71	♂		76	+	Diabetes Parkinsonism
O W	70	♂		78	+	Carcinoma of prostate
Mean	60			81		

From data of Ferry Schwartz Hager and Schroeder

NOTE The iodine number of human depot fat is 64 (348) and cholesterol is 65 8 The mean iodine number of fatty acids in plasma of normal Chinese is 156 6 (8)

TABLE XLIV

COMPARISON OF DESIGNATIONS OF LIPOPROTEINS SEPARATED BY VARIOUS TECHNICS*

Ultracentrifuge		*Electro phoresis*	*Cohn Method Fraction 10*	*Barr Russ & Eder*
Solvent Density				
1 063 (Gofman *et al*)	1 21 (Lewis Green & Page)			
Symbol				
S_f	$-S_{1\ 21}$			
20–100+	>70			
10–20	40–70			
3–8	25–40	Beta Globulin	I III	C
1–3	20–25	Alpha—2		
	2–8	Alpha—1	IV V VI	A

Note that the use of a solvent density of 1 063 does not permit alpha 1 lipoprotein to undergo flotation It is not certain which Cohn fraction contains the lipoprotein identified ultracentrifugally as alpha 2

* From Furman (352)

best, these molecules are secondary invaders or carriers and probably are not as directly concerned with pathogenesis as is cholesterol and its synthesis If the total amount of cholesterol to be carried were low there would be little or none to be deposited The nature of the fatty acids in phospholipids also may be more important in atherogenesis than the total quantity We must go deeper into first causes than a consideration of carrier components

in the blood What they are and what they are made of is of the greatest importance

The lipoproteins carry steroid hormones cholesterol and its esters, carotene or vitamin A α tocopherol and acetal lipid (containing hydroxyl groups) (352 353 387) Most (75 per cent) of the free cholesterol in serum is in the β-lipoprotein fraction as is the esterified fraction (73 per cent) while less (55 per cent) of the phosphorus is in this fraction Barr found somewhat lower values in β-lipoproteins (354 355) Thus increase in the cholesterol phospholipid ratio suspected to be of atherogenic significance means in terms of lipoproteins that with relatively less phospholipid than cholesterol the β-lipoprotein fraction will be increased in the proportion of 1 1 35 Gofman finds that particles of the S_f 10 20 classes have weight ratios of cholesterol to phospholipid as high as 1 1 3 (356)

Exogenous Cholesterol There is no evidence that a diet containing reasonable amounts of cholesterol (up to 1 0 Gm per day or the equivalent of four eggs) influences the level of blood cholesterol (357) Feeding healthy volunteers (358) or patients (359) up to ten times that amount causes insignificant changes in plasma levels * Actually at 200 mg per 100 ml blood there is about 8 Gm in circulation with an additional 3 to 4 Gm in liver and a considerable amount in other tissues While exogenous cholesterol probably little affects plasma levels in man the reverse i e restricting the dietary intake to very low values does decrease plasma levels since all dietary cholesterol is contained in fatty foods which therefore need

* It is possible to block some of the intestinal absorption of exogenous cholesterol by plant sterols such as sitosterol in large doses The effect on plasma cholesterol however is either insignificant, or significant to a very minor degree

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Solvent Density				
1.063 (Gofman *et al.*)	1.21 (Lewis Green & Page)			
Symbol				
S_f	$-S_{1.21}$			
20–100+	>70			
10–20	40–70			
3–8	25–40	Beta Globulin	I III	C
1–3	20–25	Alpha—2		
	2–8	Alpha—1	IV V VI	A

Note that the use of a solvent density of 1.063 does not permit alpha 1 lipoprotein to undergo flotation It is not certain which Cohn fraction contains the lipoprotein identified ultracentrifugally as alpha 2

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usually contain unsaturated fatty acids This is not true of fish oils which must be largely unsaturated or short chain because of the low melting point essential for mobility of the animal at low temperatures Actually fish fat contains several very long chain unsaturated fatty acids

There are three habits largely common to the U S A and some European countries which tend to raise the dietary intake of saturated fats (202 348 360) 1) Since 1920 animals fattened for slaughter have been fed high carbohydrate diets in order to lay down a hard fat Meat from animals eating unsaturated vegetable fats is oily and housewives do not like to buy it the melting point is low 2) For many years vegetable fats (unsaturated) have been commercially hardened often by a copper or nickel cata lyst in order to provide shortenings or margarine which are solid at room temperatures 3) The consumption of milk butter and cheese has increased milk fats contain shorter chain saturated fatty acids and are believed to be atherogenic (356)

Relation of Dietary Fats to Cholesterol Why does an excessive intake of hard or saturated fats cause atherosclerosis? The following information is known

1 Animals lay down in their tissues part of the fat in gested This has been demonstrated in all mammals but man Pigs fed very long chain high melting point fatty acids may crack in the cold Unnatural fats (odd numbered carbon atoms or optical isomers of natural fats) can be recovered from the bodies of animals to which they are fed in amounts from 10 to 25 per cent (348)

2 Cholesterol esters can be formed of the type of fat in the diet Thus stearic or even unnatural fatty acid esters of cholesterol can be recovered when a specific fat is fed (348)

3 The esters of cholesterol in blood usually contain

be severely restricted In animals however, especially rats rabbits, monkeys and chickens very high intakes (2 to 4 per cent of the diet) raise plasma levels markedly (356)

Type of Fat Ingested The weight of evidence at present is in favor of the idea that a diet high in fats of animal origin is atherogenic, while diets containing adequate but relatively smaller amounts of vegetable fat are not Although there are many types of fat in both animal and vegetable sources, the number is somewhat limited by the digestibility of the fats with higher melting points Natural fats contain an even number of carbon atoms and are usually found as triglycerides, the lengths of the chains vary from 4 to 24 Some are saturated, some contain one, two three or four unsaturated ethylene linkages Obviously an enormous number of possible combinations can occur fortunately, in nature they do not (Table XLIV)

The melting points of the saturated fatty acids are directly proportional to the length of the carbon chain short chain acids being liquid (348) Equal length unsaturated acids have lower melting points Solubilities are directly related the saturated acids being less soluble in water and alcohol Furthermore, the specific gravities of saturated fatty acids are lower than their unsaturated relatives

Insofar as is known there is no difference between glyceryl tristearate obtained from animal sources and that derived from vegetable the fatty acids are identical Fully hydrogenated linolenic acid becomes stearic acid What then are the differences? Unless vegetable oils contain some esoteric product which counteracts the atherogenic influence of animal fats we must look to the composition of the fats themselves In general animal fats have more saturated fatty acids than vegetable fats while the latter

usually contain unsaturated fatty acids This is not true of fish oils which must be largely unsaturated or short chain because of the low melting point essential for mobility of the animal at low temperatures Actually fish fat contains several very long chain unsaturated fatty acids

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3 The esters of cholesterol in blood usually contain

more unsaturated fatty acids than do neutral fats or phospholipids (360)

4 The metabolism of cholesterol and that of the es sential fatty acids is closely interrelated Essential fatty acid deficiency in rats is accompanied by storage of cho lesterol in liver and adrenal Diets low in fats may lower blood cholesterol but increase that in liver Linoleate or linolenate either may be required to mobilize depots of cholesterol or be necessary for its catabolism (360, 361)

5 Blood and liver levels of cholesterol in rats are ele vated in essential fatty acid deficiency when cholesterol is fed Methyl linoleate lowers both values (360)

Cholesterol is synthesized in the liver adrenal and pre sumably other tissues from acetate The possible pathways are shown in Figure 19 *Obviously it is impossible to avoid* acetate which is a metabolic product of fat carbohydrate and amino acid metabolism The immediate precursor of

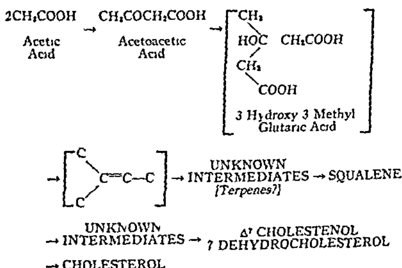


Fig 19 Possible pathway of cholesterol synthesis Entirely hypotheti cal intermediates are enclosed in brackets (From Langdon In *Fat Metabolism*, V A Najjar ed. Baltimore Johns Hopkins Press 1954)

TABLE XLV

COMPOSITIONS OF CORN COTTONSEED OLIVE OILS AND BUTTER*

	Corn	*Cottonseed*	*Olive*	*Butter*
Iodine number	103–129	90–110	79–90	26–38
Saturated acids (%)	12–18	21–32	9–19	30–43
Oleate	21–49	19–36	64–86	28–41
Linoleate	34–61	34–56	4–15	
Linolenate	0–2 9	0	0	0
Arachidonate	0	0	0	0
Squalene (mg %)	28	8	383	0
Ergosterol	+	+	0	+
Sitosterol	+	+	0	
Stigmasterol	+			
Rate of enzymic hydrolysis	1	2	3	
Tocopherol (mg %)	87–250	83–110	3–30	

* After Deuel (348) and Eckey (447) Contents vary with climate soil and seed

cholesterol has been shown to be squalene a hydrocarbon with six double bonds having the empirical formula $C_{30}H_{50}$ (362) Squalene is found in the unsaponifiable fraction of several but not all fish oils and only one plant oil olive oil (0 41 0 54 per cent) Other vegetable oils contain only very small amounts (peanut oil 0 07 per cent) Animals fed labelled squalene synthesize cholesterol 50 times as efficiently as when given labelled acetate (362) Squalene is not converted to fatty acids as is acetate An other precursor of cholesterol is provitamin D_3 or 7-dehy drocholesterol widespread in foods but in small quantities

Effect of Various Fats on Blood Cholesterol Levels in Man One can gain some information on the relationship of the type of fat ingested to the level of cholesterol in plasma by human experiments in which dietary fat was markedly increased (Table XLVI) If these results are valid an examination of the table points at once to specific dietary factors or the lack of them which alter cholesterol

TABLE XLVI

DIETARY FACTORS ALTERING PLASMA CHOLESTEROL IN MAN*

Factors Causing Increased Levels	*Factors Causing Decreased Levels*
High fat intake	Low fat intake (386)
Starvation	Scurvy
Low carbohydrate intake	Vegetable fat (Diabetics)
Vitamin B deficiency	Soy bean oil (446)
Vitamin B_6 deficiency (monkey)	Sunflower seed oil (448)
Olive oil	Nuts (363 365)
Cottonseed oil	Corn oil (367 368)
Butter (368)	Brain extract (366)

No change caused by
Obesity
Cholesterol intake (357) (moderate)

* After Deuel (360) and others

levels in man Vitamin B deficiency starvation low carbohydrate intake high fat intake olive and cottonseed oils and butter move cholesterol values in the same direction, up while specific factors appear among those reducing it Because corn and olive oils act oppositely we must look to differences in content of specific factors in these two fats

The outstanding difference appears in the linolenic content which is absent in olive and cottonseed oils* and is present to the amount of 0 6 per cent of corn oil This essential fatty acid is not found in butter unless it is fed to the cow it was present in one sample of American human fat and serum (348) (possibly influenced by diet) but was not found in one German (348) is in some lecithins and cannot be synthesized by the animal (Table

* Oils may vary in their atherogenic properties possibly because of variations in essential fatty acid (linolenate?) content (445) Olive oil consumed in Spain is usually adulterated with soy oil

XLVII) Arachidonic acid a normal component of animal tissues lecithins cephalin phosphatides and fats is another essential fatty acid formed from linoleic probably by a vitamin B_6 enzyme system (349)

Therefore the factor in certain vegetable fats (and not others) which lowers blood cholesterol may not lie in the presence or absence of unsaturated fatty acids themselves

TABLE XLVII

ESSENTIAL FATTY ACID CONTENT OF SOME EDIBLE OILS (%)

Food	*Linoleic*	*Linolenic*	*Production*†
Linseed	15–43	40–53	2 2
Peanut	47–72	0	3 9
Sunflower	44–75	0 1	2 0
Sesame	40–48	0	1 5
Soy bean	52 0	2 3–11	3 8
Coconut	1–2	0	4 6
Animal fats	+	0	17 8
Rapeseed	12–16	7–10	3.3
Palm	6–11	0	3.3

* After Eckey (447) †Estimated World 1951 billions of lbs

but in the linolenic or other specific fatty acid content The cholesterol lowering diets of Kinsell *et al* contain nuts in large amounts (363) The fat from some nuts especially walnuts contains linoleic and linolenic acids (348) In this respect Kinsell s diets (364 365) contained soybeans soy lecithins soy sauce corn oil and walnuts all containing linolenic acid while this fatty acid has not been found in peanuts almonds and cashew nuts (348) The hydrogenated oils in margarine Crisco and peanut butter as well as cottonseed peanut and olive oil which apparently do not contain linolenate were also given in spite of these fats at least two of which usually raise blood cholesterol it fell Brain extract probably cephalin which

contains arachidonic acid also has been reported to lower cholesterol (366) The common denominator of the effects of these various conflicting data appears to be in the tri or tetraethenoid acids similar to linolenic and arachidonic (367 369) (Table XLVIII)

TABLE XLVIII

FOODS CONTAINING LINOLEIC LINOLENIC AND ARACHIDONIC ACIDS*

	Linoleic (*C_{18}, 2 double bonds*)	*Linolenic* (*C_{18} 3 double bonds*)	*Arachidonic* (*C_{20} 4 double bonds*)
Egg yolk lecithin	+	0†	+
Brain lecithin	0	0	+
Brain cephalin		0	+
Liver lecithin	0	0	+
Pig fat	+	0	2 1%
Butter fat	3 6–4 5%	?**	+
Fowl fat	21 3%	0	+
Fish	40±%	+	+
Soy bean lecithin	+	+	0
Phosphatidic acids	+	+	0
Walnut oil	73%	3–8%	0
Beechnut oil	38%	0 4–2 9%	0
Soy bean	52 0%	2 3%	0
Alfalfa	67 5%	20 8%	0

* After Deuel (348)
† Only when fed to hens
** *Only when fed to cows*

It is interesting that atherosclerosis is unusual in countries where soy beans are a staple article of diet If these deductions are correct substances containing linolenic acid, either in fats or in phosphatides, should lower blood cholesterol * The relationship between pyridoxal and the formation of linolenate will be discussed below

* The practice of fasting by some religious groups during one day a week and the Lenten season may have logic in terms of essential fatty acid

Pyridoxal and Trace Metals The amount of vitamin B_6 in the diet has a definite influence on essential fatty acid metabolism in animals Vitamin B_6 deficiency and essential fatty acid deficiency in rats resemble each other grossly and each factor will partly alleviate the other There are however fundamental differences in enzymes in the two

TABLE XLIX

COMPARISON OF ESSENTIAL FATTY ACID AND PYRIDOXINE DEFICIENCIES IN RATS (384)

Function	*Organ*	*Essential Fatty Acid*	*Pyridoxine*
Respiration	Liver	Increased	
Cytochrome oxidase	Liver	Increased	Increased ±
Succinic oxidase	Liver	No Change	No Change
Phosphate esterification	Liver	Decreased	Decreased
Glutamic dehydrogenase	Liver	Decreased	Decreased
Butyric dehydrogenase	Liver	Decreased	No Change
Succinic dehydrogenase	Liver	Decreased	No Change
Glutamic decarboxylase	Brain	—	Decreased
Arachidonic synthesis	Carcass	Decreased	Decreased
Hexaenoic synthesis	Carcass	Decreased	Decreased
Octanoate oxidation	Carcass	—	Decreased

conditions (Table XLIX) Apparently vitamin B_6 is essential for desaturating partly unsaturated fatty acids such as linoleic further to synthesize arachidonic and in metabolizing linolenic to hexaenoic acids Linoleic is a precursor of arachidonic and linolenic of the hexaenoic acids (349)

deficiency or saturated fatty acid excess Thus the members of the Russian Orthodox Church eat nothing of animal origin during Lent Advent and on Wednesdays and Fridays *and use oils high* in linolenic acid Roman Catholics by custom supply themselves with adequate essential fatty acids on Fridays and during Lent but do not restrict other animal fats Mohammedans have strict dietary laws *during Ramadan* In terms of deposition of lipid these religious habits probably do no harm in maintaining or restoring the integrity of the intima.

In deficiency of this coenzyme, liver fat becomes more saturated (370)

Snell believes that vitamin B_6 as a coenzyme contains a metal for activity (215) If so, the interrelationships are obvious although whether or not such a metalloenzyme acts in the fatty acid cycle is not known The only one of these enzymes containing a metal is acyl Co enzyme A de hydrogenase which uses copper

Some further information may be obtained by the use of metal binding and chelating agents in lowering blood cholesterol Calcium disodium ethylenediamine tetraace tate (EDTA) is a good cholesterolytic agent in man (Figs 20 21) *sometimes lowering values to the Indian normal* (180, 371, 372) In rabbits (373) and rats (374) fed cholesterol however, it raises blood levels above the controls and in rabbits but not in rats it prevents deposition of this lipid in the liver Because EDTA is not metabolized in the body and diffuses readily (250) its sparing effect on liver but not on blood levels must be related to removal of one or more trace metals EDTA also raises lipid syn thesis by rat liver while another agent 8 hydroxquinoline lessens it (375) *EDTA causes marked loss of subcutaneous* and depot fat (376) suggesting that a metal is involved in fat metabolism and synthesis Hydralazine another metal binding agent also lowers cholesterol in man (180) (Fig 22)

Comment These indirect but quite suggestive data are provocative of thought when viewed in pathogenetic and therapeutic terms Obviously normal cholesterol levels are lower in some countries than in others usually in those less touched by *Western Civilization If we could* reduce our own to these normal values by interfering *with* the processes which raise them to abnormal levels

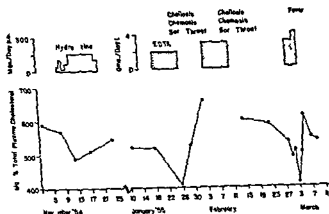
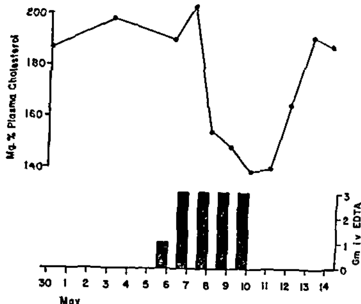


Fig. 20 Effects of oral hydralazine and intravenous EDTA on plasma cholesterol levels L. T 44 years of age was seen in October 1954 because of mild exertional dyspnea and ankle edema for six months At 7 years of age he had Marie Strumpell arthritis and at 25 years of age migratory polyarthritis without urinary symptoms Nephrosis was clinically evident and amyloidosis was proved by renal punch biopsy To lower his plasma cholesterol hydralazine was begun without evident clinical improvement. In a further effort to lower his plasma cholesterol three courses of parenteral EDTA were given On the sixth day of this first course of therapy slight inflammation of the mucous membranes and a magenta tongue were observed and he complained of soreness about his mouth and gums By the final day cheilosis chemosis scrotal inflammation and pustular lesions over the face and trunk had appeared Within a week the lesions had vanished and a second course of EDTA was begun On the fourth day stomatitis reappeared and within 7 days the same syndrome was present again necessitating the discontinuation of therapy The 3-day final course produced no such lesions however fever immediately followed the dosage increase to 4 Gm. Cholesterol values for the second course were not plotted because such a low plasma level was attained that a laboratory error was suspected (303 mg per 100 ml on a single determination) The changes in cholesterol preceded clinical toxicity (From Perry H M Jr and Schroeder H A J Chronic Dis 2 520 1955) Metal excretion in Table XXX p 160



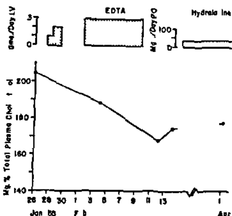




Fig 21 Effect of intravenous EDTA on plasma cholesterol The material was given as the calcium disodium chelate L S The patient s diabetes controlled on 45 units of insulin a day worsened to the point that it needed 55 units possibly a result of pancreatic loss of zinc (From Perry H M Jr and Schroeder H A *J Chronic Dis* 2 520 1955) A M Hydralazine given in small doses appeared to maintain the lower values achieved with EDTA M B The rebound in a month is obvious

we would expect little or no atherosclerosis especially of our coronary arteries

There appears to be something in certain but not all vegetable fats which lowers blood cholesterol markedly in man while animal fats hydrogenated vegetable fats and other vegetable fats with a lower iodine number raise plasma cholesterol As a first guess this substance may be linolenate an essential fatty acid If that is so Europeans and Americans may be suffering from a relative essential

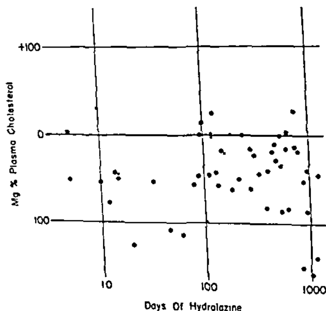
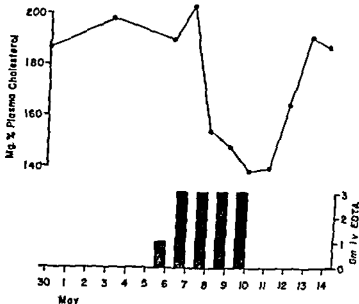


Fig 92 Effect of oral hydralazine on total fasting plasma cholesterol in 119 hypertensive patients. Changes in cholesterol concentrations before and after hydralazine are plotted against the length of therapy Each large dot indicates a patient with an initial cholesterol level of more than 215 mg per 100 ml plasma. Each small circle indicates a patient with lower initial values below 211 mg per 100 ml plasma (From Perry H M Jr., and Schroeder H A J Chronic Dis 2 590 1955)



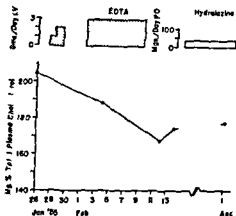




Fig 21 Effect of intravenous EDTA on plasma cholesterol The material was given as the calcium disodium chelate L S The patient s diabetes controlled on 45 units of insulin a day worsened to the point that it needed 55 units possibly a result of pancreatic loss of zinc (From Perry H M Jr and Schroeder, H A *J Chronic Dis*, 2 520 1955) *A M Hydralazine given in small doses appeared to* maintain the lower values achieved with EDTA M B The rebound in a month is obvious

×	Intimal Injury due to a) Vitamin B deficiency b) Excessive hypertension c) Normal pressure differentials	×	Increased Saturated over Unsaturated Fatty acid esters of cholesterol lipoproteins and phospholipids Increased synthesis or decreased destruction of cholesterol due to metals or fatty acid deficiency	×	Blood Pressure	= Atherosclerosis

The nature of the vitamin B_6 deficiency and its possible relation to trace metals has already been discussed The plasma lipids under consideration include a) Cholesterol esters which are said to be usually of unsaturated fatty acids although esters of equal length saturated fatty acids are lighter and more insoluble Dietary excess of the latter could influence their nature b) Lipoproteins or protein fatty acid complexes the nature of which are unknown The saturated fatty acid esters should be lighter and more insoluble than their unsaturated counterparts If so they should centrifuge more slowly (or float more rapidly) A lipoprotein with a specific flotation rate of S_f 0 10 if its fatty acid became saturated should theoretically change to the S_f 10 20 class unless rearrangement of the molecule took place c) Phospholipids the fatty acid components of which depend partly perhaps on dietary intake but usually are formed of unsaturated fatty acids in functioning tissue (brain and liver)

Effect of Sex For some unknown reason premenopausal women are quite immune from coronary atherosclerosis The disorder however becomes as frequent in women after the menopause as in men Coronary occlusion in a normotensive menstruating woman was formerly extremely rare although cases are now appearing The degree of aortic atherosclerosis however shows little sex

fatty acid deficiency There may be very little linolenic acid in the American diet Pyridoxal is concerned with the utilization of essential fatty acids and a deficiency of one may enhance a deficiency of the other Furthermore, trace metals can be involved since they affect fat synthesis and may be concerned in vitamin B_6 enzymes although their role is possibly of only secondary importance

The differential effects of metal chelating agents must be explained Let us assume that in the livers of human beings there is a metal (chromium, for example) which stimulates the formation of cholesterol and fatty acids or a metal (copper for example) which depresses catabolism of lipids, in addition to the normal metal (manganese *for example) EDTA because of its higher affinity for the* abnormal metal removes it allowing synthesis *to revert from an accelerated to a normal rate or catabolism to* raise itself to normal In the animal fed cholesterol however there is no *abnormal metal affecting synthesis* therefore the normal one is removed In that event the synthesis of cholesterol in liver might be lowered or catabolism raised preventing accumulation of endogenous cholesterol in liver but allowing exogenous cholesterol to remain in the blood The latter would be the case if storage and synthesis were related The differential effects of EDTA and 8 hydroxyquinoline in the rat can be explained by different affinities of the two substances for metals the former being the stronger chelating agent *That two an*tagonistic metals may influence an enzyme system is well *known for actomyosin (Mg and Ca) and has been proposed* by the Bernheims for lipid metabolism (Mn and V) (274)

These various factors can be substituted in Friedman s schema to include a more definitive but much more hypothetical one

fluctuations can occur (377) A persistently high level is atherogenic a momentary low level may not reflect the true state of affairs in terms of intimal exposure There are conflicting opinions and data but the opposing views can be resolved by realization that a) cholesterol levels fluctuate and unless consistently elevated values may be meaningless 2) when a patient is sick the levels fall 3) the full lesions of atherosclerosis develop only after prolonged constant or intermittent hypercholesterolemia

In this chapter we have spoken of trace metal imbalances conditioned vitamin B_6 deficiencies and essential fatty acid deficiencies We have emphasized that these deficiencies are relative conditioned and local to one or at most a few enzyme systems There is no practical way however of reversing vitamin B_6 deficiencies at the present time The administration of 50 mg of pyridoxal hydrochloride daily to many patients has not resulted in a detectable fall in blood cholesterol The administration of at least two trace metals cobalt and manganese in large daily doses have not caused clinical changes detectable by ordinary laboratory methods Only by chelating agents have we been able to affect blood levels favorably (180)

The several pathogenetic factors outlined by Friedman *et al* should be affected simultaneously if we are to expect cessation of the process or at the best reversal Whatever is making the intima injured so that plaques are formed should be opposed as an approximation pyridoxine in adequate doses is required until more is known The abnormally high cholesterol levels in blood should be reduced by dietary influences and chelating agents if possible Elevated diastolic pressure should be controlled at normotensive levels Under these conditions some reabsorption of plaques which are not too scarred might be expected

difference (355) These differences cannot be explained by gross differences in plasma lipids, cholesterol or phospholipids but lower concentrations of beta lipoproteins higher ones of alpha lipoproteins and lower atherogenic ultra centrifugal lipoproteins are found in women The administration of estrogens to men alters these values to those of healthy young women, while methyl testosterone acts in the opposite direction Estrogen shifts an appreciable amount of cholesterol carried by beta lipoproteins into alpha lipoproteins while testosterone does the opposite The same sex immunity to coronary disease is found in chickens Although estrogen is carried in blood by lipoproteins this phenomenon is unexplained on a mass action basis by the small amounts administered

Clearing Factor Heparin will clear lipemic serum both *in vivo* and *in vitro* (444) Apparently this anticoagulant alters the physicochemical structure of chylomicra so that they become soluble These large fat filled particles carry almost all *dietary cholesterol* The rates of clearing are faster in young women than in men and slower in the aged Their relations to atherosclerosis are not known

CLINICAL IMPLICATIONS

At present there are several available methods for lowering blood cholesterol in man Because the pathogenesis of atherosclerosis is a multi valent one the process must be attacked at different levels Obtaining a permanently lowered cholesterol level in blood might allow cessation of the deposition of cholesterol in plaques with probably some absorption in the presence of an altered gradient between plaque and plasma Practical methods will be discussed in Chapter IX

What the level is at any one instant of a lifetime of exposure to atherosclerosis is relatively unimportant Wide

3 Ischemia due to organic vascular disease which does not appear until the pressure is lowered is a clear and present hazard in actual practice it is rare

4 Diastolic normotension must be achieved and maintained whenever possible A compromise is hazardous merely modifies the disease promotes drug tolerance and does not allow eventual reduction of dosage

5 The use of any ganglionic blocking agent the action of which does not last for 24 hours requires that blood pressure be measured before each dose in order to prevent a) hypertension b) hypotension and c) to provide as constant a blood level as possible throughout the day and night Varying requirements and absorption necessitate varying dosages according to the prevailing levels of blood pressure

6 Arterial hypertension due to increased generalized vasospasm is a disorder or a disease The patient either has it or has not If he has severity varies widely from slight to marked Therapy should be applied when both patient and physician want to control the disease If therapy is not applied the responsibility rests on the physician that the disease is not doing or going to do harm

EVALUATION OF PATIENT FOR DRUG THERAPY

The first question to be answered is Has the patient hypertension? A diastolic pressure of 90 mm Hg or over (measured by the disappearance of Korotkoff sounds) is strongly suggestive in fact usually indicative of generalized vasospasm in the absence of tachycardia polycythemia or coarctation of the aorta When persistent it suggests chronic hypertension when relieved by relaxation it suggests the prehypertensive state

The second question is How severe or sustained is the

Chapter VIII

PRACTICAL METHODS FOR MODERN THERAPY OF HYPERTENSION

INTRODUCTION

On the basis of the various hypotheses and findings outlined in previous chapters and using the potent drugs discussed a practical method of controlling excessive vasospasm in man can be outlined and the therapeutic limits of a long term regimen can be predicted While the use of this regimen requires care and precautionary measures, it is no more difficult in fact much less so than is the control of diabetes mellitus Some simple but basic rules need be kept in mind, which any practitioner of medicine can follow The results are often life saving in severe cases

General Rules 1 Vasospasm alone is being treated *In no case are the results of atherosclerosis (coronary and cerebral arterial narrowing the loss of aortic elasticity,* renal arteriosclerosis) being reversed as far as we know, although there may be slow changes with time Therefore the most striking results will be seen in cases where the effects of vasospasm are the greatest (hypertensive heart failure cerebral edema), and the poorest in cases where the effects of atherosclerosis are the most advanced

2 In all cases where two different factors are contributing to the generalized vasospasm two differently acting drugs must be used When only one factor is operating, only one drug is necessary In severe hypertension two factors almost always are functioning

primary organic renal diseases adrenal dysfunction or emotional crises

For a thorough work up evaluation of the cardiac status requires a study of the symptoms electrocardiographic tracing for left ventricular hypertrophy strain patterns or old myocardial infarction and roentgen or fluoroscopic examination of the heart In actual practice only signs of a previous coronary occlusion are important indications for cautious therapy but physicians are often gratified to watch enlarged hearts slowly become smaller and abnormal electrocardiographic tracings revert to normal under continuous therapy

Renal status is evaluated most easily by routine urinalysis and the intravenous injection of phenol red (PSP) with urine specimens obtained 15 30 and 60 minutes after injection The test is simple and reliable The bladder need not be emptied before the test although the first specimen may show a somewhat smaller amount of PSP * for unknown reasons (Reabsorption of PSP by bladder wall has not been ruled out) Adequate hydration is essential for accurate values The urinary concentration test is impractical on an outpatient basis owing to difficulties in restricting the amount of dietary water If the 15-minute PSP excretion is less than 10 to 15 per cent azotemia may be suspected

Retinoscopy is essential in all cases The appearance of exudative or hemorrhagic retinitis is often a poor prognostic sign making treatment mandatory The best method for grading fundal changes is that of Keith and Wagener

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* In a series of 39 medical students paired for the test with bladder empty the 15-minute excretion was 35.5 per cent with bladder full, 30 9 per cent The normal values with bladder initially empty were 1 minutes 35.5 per cent 30 minutes 21.2 per cent 60 minutes 17.0 per cent with a total of 73 7 per cent

hypertension now? This can be answered only by repeated measurements of blood pressure sometimes complete bed *rest is necessary to rule out emotionally induced vasospasm* The examining physician must be aware of the mano metric reflex, ' a psychosomatic response by vasospasm to the wrapping of the cuff about the arm (4) He also must keep in mind the wide spontaneous variations of blood pressure sometimes seen in hypertensive individuals (388 37, 36)

The third question is How much harm, if any, is hy pertension doing or has it done? This can be answered mainly by physical examinations and simple laboratory tests, bearing in mind *the sharp division of what changes* are atherosclerotic and what are the results of overwork and vasospasm Systolic hypertension is not caused by vasospasm but by hard arteries or increased cardiac out put, cerebral thrombosis and coronary occlusion are not directly hypertensive in origin

The fourth question is How rapidly is it progressing? An accurate *history* and *clinical judgment* supplies the answer, often only after many examinations

Office Practice While partial answers to these questions can be obtained in office practice one can do better by *a hospital work up (400)*

The date of onset of hypertension should be determined *if possible in order to get an idea of the rate of progres* sion The date of discovery may be less important The *effects of hypertension can be evaluated by inquiry into* the cardiac status, dyspnea being the earliest symptom of *insufficiency and renal status by polyuria and nocturia* The approach to the patient is divided into three parts a) *estimation of the rate of progression* b) *estimate of the* present status in terms of secondary organic damage and c) *inquiry into possible definite etiologic factors such as*

5 External compression of the renal artery
  a Tumors of the pedicle*
  b Aneurysms*
6 Diminution of the calibre of the renal arteries
  a Congenital malformations hypoplasia*
  b Atherosclerosis, with atheroma of the main renal artery (common)*
7 Disorders of the urinary tract
  a. Obstructive disorders
    (1) Lithiasis*
    (2) Hydronephrosis (*usually infected*)*
    (3) Pyonephrosis
    (4) Congenital malformations*
    (5) Prostatic hypertrophy*
    (6) Uterine prolapse
    (7) Pelvic tumors (fibromyomata)*
  b Pyelonephritis (common)*
8 Venous obstruction
  a External compression of renal vein
  b Congestive heart failure*

The following intrarenal diseases must be considered

1 Inflammatory vascular lesions
  a Disseminated lupus erythematosus*
  b Polyarteritis nodosa*
  c. Syphilis
  d Thromboangiitis obliterans
2 Inflammatory renal lesions
  a Glomerulonephritis*

The following endocrine diseases can influence hypertension

1 Hypophyseal tumors and hyperfunction*
2 Adrenal cortical and medullary tumors and hyperplasia*

(389), in simplest terms it means Grade I, spasm, Grade II, spasm and sclerosis Grade III, spasm and hemorrhage and/or exudate, Grade IV, spasm, hemorrhage and/or exudate plus papilledema Cases between grades are naturally encountered

The lability of the blood pressure can be tested by giving tetra ethylammonium chloride (Etamon) intravenously, thus blocking sympathetic ganglia This drug is not to be given in states of renal insufficiency excreted by the kidney, it may be retained and the patient may suffer from postural hypotension for several hours

Intravenous pyelography and repeated cultures of the urine are essential for revealing the presence of chronic pyelonephritis if active low grade infection is present Estimation of the numbers of bacteria per ml of urine (colony count) is of more value than merely finding organisms

The following renal conditions usually discovered by pyelography or aortic arteriography, can influence hypertension unfavorably by adding a renal factor to a neurogenic one The list was modified from that of Braun Menendez *et al* (148, 393, 394)

1 Reduction of renal parenchyma
 a Polycystic kidneys*
 b Renal tumors (rare)
 c Hydatid cyst of kidney (rare)
 d Traumatic lesions*
 e *Hypoplasia**
2 Perinephritis, healed*
3 *Complete obstruction of main artery or branch**
 a Thrombosis and atheromata of the renal artery*
 b Emboli to the renal artery clot or cholesterol*
4 Intermittent occlusion of the renal artery
 a Renal ptosis*

* Personally observed

EVALUATION OF GENERALIZED VASOSPASM IN HYPERTENSIVE STATES

It is of little more than academic interest what the underlying pathogenetic factors in a state of severe hypertension may be except when chronic pyelonephritis can be treated with antibiotics (often with little success) or recurrences of glomerulonephritis prevented by antibiotics designed to abort upper respiratory tract infections It also matters little what the type of renal disease contributing to the vasospasm may be pyelonephritis glomerulonephritis secondary arteriolar nephrosclerosis or even polycystic disease What do matter are the relative influences of neurogenic nephrogenic or adrenocortical factors in causing the vasospasm for the relative amounts of different drugs required will differ according to the amount of renal ischemia present Therefore it is a good plan to group cases according to several stages of the disease dependent upon the amount of vasospasm one finds and its lability

The degree of lability of the vasospasm is the factor which determines these stages Complications such as cerebral vascular accident and coronary arterial occlusion can occur in any stage mild severe or normotensive as they are caused not directly by vasospasm but by an associated disease atherosclerosis The fact that this other disease can be influenced by the severity of the hypertension i e the vasospasm has little to do with therapeutic measures aimed at vasospasm Therefore classifications based partly upon atherosclerotic damage are valid for purposes other than the choice of drugs or procedures such as prognostic implications and for surgical risk One would not use the most potent drugs in a patient with hemiplegia or congestive heart failure who exhibited severe atherosclerosis and mild hypertension one would use them however in a patient with severe but asympto-

3 Ovarian tumors*
4 Toxemia of pregnancy*
5 Testicular tumors with hyperfunction*

The following nervous lesions can contribute to hyper tension

1 Certain tumors of brain*
2 Anxiety states*
3 Expanding inflammatory lesions*
4 Cerebral vascular lesions*

Hospital Patients For further evaluation, the patient should be examined in hospital both for the purpose of determining the lability of blood pressure and for initiating treatment with ganglionic blocking agents Blood pressure is measured every 4 hours by the nurses and is charted The sodium amytal release test is performed (0 2 Gm sodium amytal given each hour for 3 hours, blood pressure being measured hourly during the night) The ability of the kidneys to concentrate urine also can be measured by giving the patient a dry diet and no fluids for 12 18 or 24 hours the last specimen shows the maximal specific gravity corrected for proteinuria or glycosuria

The obvious disorders causing true or false hypertension must be ruled out before starting therapy Pheochromocytoma is a rare disease requiring a high index of suspicion, it can be suspected by using phentolamine (Regitine) intravenously without prior sedation Regitine can cause acute hypotension and renal shut down in azotemic states Measurement of catechol amines in urine either by bioassay (390) or chemical determination, is a specialized procedure done only in a few medical centers Coarctation of the aorta is unusual palpation of the femoral arteries or abdominal aorta will usually show its presence as will roentgenograms of the rib cage

Serious secondary atherosclerotic complications may or may not be present if so about 30 to 40 per cent may be dead in 3 years The ocular fundi are Grade I or II renal function is normal or nearly normal and the blood pressure is roughly 180/100 to 220/120 mm Hg during rest in bed Reserpine plus fairly large doses of hydralazine (300 to 400 mg per day) will control about half of these cases eventually the remainder require the addition of ganglionic blockade With time individuals in this stage uniformly exhibit reversal of the process and marked reduction of dosage in 2 to 3 years a majority can be maintained on reserpine alone and a few will be in a complete but probably temporary remission

Stage III is made up of individuals with Grade II to III (Keith Wagener) ocular fundi with or without occasional hemorrhagic and exudative lesions with severe generalized vasospasm and hypertension not relieved by heavy sedation (sodium amytal) Renal function is adequate but usually reduced Serious atherosclerotic complications may or may not be present. The blood pressure is usually 200/120 to 270/160 mm during rest in bed In this stage which usually carries a poor prognosis (40 per cent dead in 3 years) ganglionic blockade plus adequate doses of hydralazine (500 mg or more per day) are essential for control Reserpine may or may not be added mainly for its sedative action in smoothing out variations in blood pressure caused apparently by emotional lability in the presence of incomplete and irregular ganglionic blockade Therapeutic results are good 95 per cent surviving 5 years with considerable eventual reduction in dosages in most

Stage IV corresponds to Perera s accelerated phase or what is more commonly called the malignant stage For therapeutic purposes it can be divided into three sub-

matic diastolic hypertension and retinitis without serious cardiovascular damage having yet occurred

We define normotension as levels below 140/90 mm Hg It is at this diastolic level that vasospasm becomes slightly excessive Definitions based on higher diastolic levels in older age groups pay lip service to the prevalence of the disease, avoiding realities Actually in the absence of vasospasm the diastolic pressure falls slightly as atherosclerosis develops while the systolic rises

Prehypertensive The blood pressure is slightly above 140 mm systolic or 90 mm Hg diastolic at times but falls with relaxation There are no signs of secondary damage Chronic pyelonephritis or hydronephrosis, however, may be found in young individuals (391)

Stage I comprises patients with elevated levels of blood pressure during a physician s examination but normal or lower levels part of the time corresponding to early stages of Perera s uncomplicated asymptomatic phase (392) Admission to hospital and frequent measurements by nurses combined with diagnostic procedures lower them to normal, often dramatically (400) Renal function is excellent and there are no findings suggestive of secondary damage Emotional tension is present and continuous Reserpine will effectively control about half of these cases in the remainder small doses of hydralazine (75 to 200 mg per day) may be necessary We do not advocate the use of protoveratrine or available blocking agents in these individuals, for their irregularity and intermittency of action may cause hypotensive symptoms A very long acting ganglionic blocking agent however should be effective

Stage II comprises patients with elevated levels of blood pressure at all times except under heavy sedation (sodium amytal) It corresponds partly to Perera s symptomatic uncomplicated phase and partly to his complicated phase

more theoretical benefits of dividing the dose or of using slowly absorbed increments such as are dispensed in some modern capsules than there is in dividing the dose of digitalis It has been our practice to use 1 0 mg per day for 1 month reducing it then to 0 5 mg and further reducing it to 0 25 or 0 1 mg slowly if excessive sedation appeared It is such a mild anti hypertensive drug in most cases that it should not be depended upon if signs of cardiovascular damage are present Its many side effects are listed in Chapter III nasal congestion being the most prominent and paranoid depression with suicidal tendencies the most dangerous This latter side effect places it among the agents indirectly hazardous to life the self satisfaction of a physician using it in severe and malignant stages who is afraid to employ more potent measures also makes it less directly hazardous At the first sign of *increasing* nervousness anxiety insomnia emotional tension and agitation it should be discontinued for agitated paranoid depression may develop (70 72) In our experience reserpine is not a very valuable agent for hypertension *except* as a curious kind of sedative with many side effects (*Fig* 24)

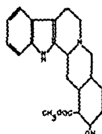
Reduction in dosage will often alleviate the usual depressive effects of the agent and may reduce the number of nightmares but nasal stuffiness can be most annoying Epistaxis induced by the drug usually necessitates discontinuation The time tested sedatives are the only alternatives to replace reserpine if it cannot be used

We have seen four patients whose severe hypertension was cured for several months in that all drugs but reserpine were slowly discontinued during 2 to 3 years In one an ulcer developed in two inactive ulcers became active one having a massive hemorrhage and perforation into the pancreas requiring partial gastrectomy in the fourth

stages (a) early, in which hemorrhagic and exudative retinitis is present but not marked (Grade III to IV) and renal function is reduced but adequate, (b) severe, in which retinitis is advanced (Grade IV) and renal function is borderline, and (c) azotemic in which nitrogen retention has occurred The diastolic pressure is usually 130 mm or more and fixed and albuminuria is usually present Each sub stage carries a different prognosis, 3 year survivals of treated patients being roughly 100 per cent for (a), 80 per cent for (b) and 50 per cent for (c) without frank uremia Ganglionic blockade plus adequate doses of hydralazine (500 to 1000 mg per day) are essential for reversal of the stage In general control of hypertension is easier to achieve and is more even than with patients in Stage III, reduction of dosage is the rule after 12 to 18 months except in azotemic individuals The systolic pressure can usually be maintained at or near former diastolic levels

SPECIFIC USE OF DRUGS

Use of Reserpine Reserpine is given in one dose a day usually at night (Fig 23) Since the effect of this drug is cumulative (although the drug itself is not), there are no



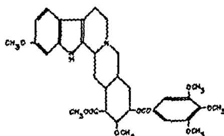


FIG 23 Chemical structures of Yohimbine and Reserpine according to Schlittler *et al* (442)

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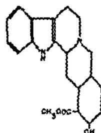
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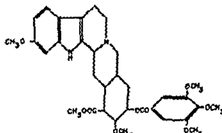
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YOHIMBINE RESERPINE

Fig 23 Chemical structures of Yohimbine and Reserpine according to Schlittler *et al* (442)

serious mucous colitis and proctitis with ulceration and bleeding appeared and remained Because reserpine produces relative parasympathetic overactivity it may be con traindicated in such individuals * and complications such as these must be watched for

Use of Protoveratrine One can begin this agent in doses of 0 2 mg three times a day increasing by 0 2 mg per dose until nausea or vomiting appears (395) The dose causing nausea is then reduced by 0 1 or 0 2 mg and the others gradually increased to the point of nausea The emetic effect appears within an hour after the dose often sooner Wide swings of blood pressure occur with a rise at night This agent cannot be given effectively every 4 hours as tolerance soon develops only to disappear with a few hours rest To be completely effective

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These developments stimulate speculation If psychosomatic influences are blocked in one somatic nervous pathway the sympathetic perhaps excessive outflow through the other the parasympathetic, has led to gastrointestinal disorders The patient is determined to develop a psychosomatic disease when we do not allow him (or usually her) to have one kind he gets another Reserpine may thus be an accessory etiologic agent

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been on treatment for 2 years with a fair response his pretreatment levels ranging between 200 and 250 systolic and 130 and 150 mm. Hg diastolic Congestive heart failure had disappeared without drugs or dietary restrictions Note the decreased intake of hexamethonium chloride required when reserpine was added the average level of blood pressure appeared to rise at end of the period. G C. same in a 54-year-old man with severe aortic atherosclerosis who after 1 year achieved only a fair response from initial levels of 205 to 240 systolic and 120 to 140 mm. Hg diastolic. He preferred to maintain his blood pressure at levels higher than normal and to vary his dose of hydralazine his diastolic pressure was only slightly elevated Note the reduction in average daily dosage of both agents without change in blood pressure when reserpine 1 0 mg a day was added. Control had been previously increased on two occasions by adequate doses (From Schroeder H A. *Am J Med* 17 540 1954)

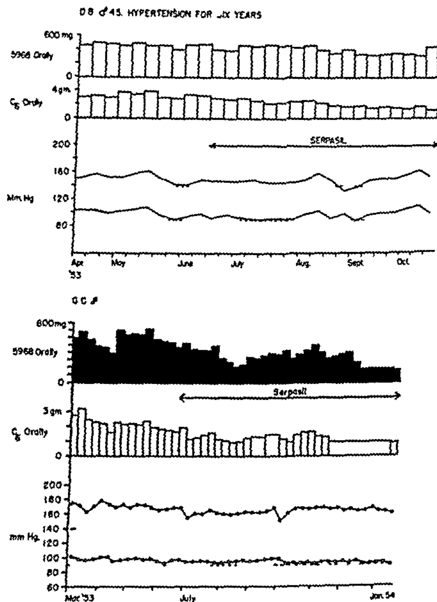


Fig 24 Effect of added reserpine D B mean weekly blood pressure levels (each 35 measurements) and average daily doses of hydralazine (5968) and hexamethonium chloride (C_6) as affected by reserpine (Serpasil) 1 0 mg per day in a patient with severe benign hyper tension previously suffering from congestive heart failure He had

to judge accurately the effective dose of a ganglionic block ing agent without knowing the level of blood pressure It is most difficult to give increasing doses to maximal effect without overdosage unless the patient is under care ful supervision of blood pressure and symptoms There fore it is our practice to begin ganglionic blockade by drugs in the hospital not only for convenience sake but also to achieve greatest benefit We know of only one way to give oral ganglionic blocking agents effectively (Fig 26)

1 Blood pressure is measured by competent nurses every 4 hours day and night and charted

2 Initial dose is given every 4 hours if the blood pres sure is above a chosen level usually 140 mm systolic It is not given if the blood pressure is below that level Slightly higher omit levels are used in the cases of atherosclerotic or azotemic patients 150 to 170 mm Hg Initial doses which are usually safe to give to patients with severe hypertension are Hexamethonium chloride 125 mg Pentolinium tartrate 20 mg Chlorisondamine 10 mg Mecamylamine 2 5 mg

3 If the desired normotension is not achieved each dose is raised by increments amounting to the initial dose daily until 4 or 5 days have passed Thus each dose given every 4 hours will be Hexamethonium chloride 500 to 750 mg Pentolinium tartrate 150 to 200 mg Chlorison damine 50 to 75 mg Mecamylamine 15 to 20 mg By this time intermittent normotension should have been achieved (In order to change intermittency to more even control hydralazine must be added at this point (Fig 27)) Each dip in systolic pressure gives the physician confi dence in the lowest levels tolerable without cardiovascular accident

4 Doses are then given on a sliding scale dependant on the level of blood pressure If normotension is desired

protoveratrine must usually be supplemented by hydralazine (Fig 25)

Use of Ganglionic Blocking Agents It is impossible

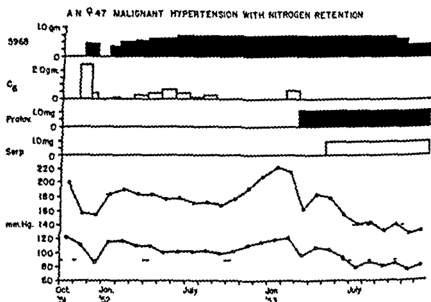


Fig 25 Example of poor initial control of blood pressure by insufficient doses and excellent control later in a patient with malignant hypertension and renal insufficiency who also suffered from partial asymptomatic duodenal obstruction Hypertension of 10 years duration had become severe and grade IV ocular fundi had developed She had suffered a minor apoplectic stroke Hexamethonium chloride induced intermittent subtotal obstruction and vomiting and was poorly tolerated after 2 months After the initial excellent response a grade of only a fair response was achieved on large doses of hydralazine 1 year later blood pressure slowly returned to control levels The addition of protoveratrine caused a sharp decline to lower levels the later addition of reserpine (serpasil) produced normotension Each point represents the average of 150 measurements Elevated nonprotein nitrogen in her blood of 40 mg per cent (Somogyi zinc method) had fallen to 16 mg per cent an abnormal electrocardiogram had become normal and her ocular fundi had cleared In our experience the effect of these two additional agents in severe stages is unusual This case is illustrative of the point that control of hypertension is possible in almost all cases (From Schroeder H A. *Am J Med*, *17* 540 1954)

to judge accurately the effective dose of a ganglionic blocking agent without knowing the level of blood pressure It is most difficult to give increasing doses to maximal effect without overdosage unless the patient is under careful supervision of blood pressure and symptoms There fore it is our practice to begin ganglionic blockade by drugs in the hospital not only for convenience sake but also to achieve greatest benefit We know of only one way to give oral ganglionic blocking agents effectively (Fig 26)

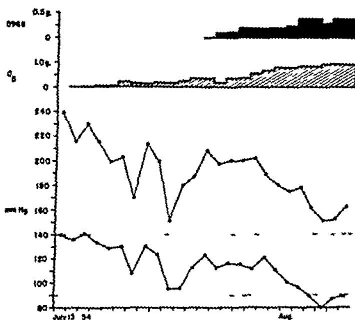
1 Blood pressure is measured by competent nurses every 4 hours day and night and charted

2 Initial dose is given every 4 hours if the blood pressure is above a chosen level usually 140 mm systolic It is not given if the blood pressure is below that level Slightly higher omit levels are used in the cases of atherosclerotic or azotemic patients 150 to 170 mm Hg Initial doses which are usually safe to give to patients with severe hypertension are Hexamethonium chloride 125 mg Pentolinium tartrate 20 mg Chlorisondamine 10 mg Mecamylamine 2 5 mg

3 If the desired normotension is not achieved each dose is raised by increments amounting to the initial dose daily until 4 or 5 days have passed Thus each dose given every 4 hours will be Hexamethonium chloride 500 to 750 mg Pentolinium tartrate 150 to 200 mg Chlorisondamine 50 to 75 mg Mecamylamine 15 to 20 mg By this time intermittent normotension should have been achieved (In order to change intermittency to more even control hydralazine must be added at this point (Fig 27)) Each dip in systolic pressure gives the physician confidence in the lowest levels tolerable without cardiovascular accident

4 Doses are then given on a sliding scale dependant on the level of blood pressure If normotension is desired

H J ♂ 61 MALIGNANT HYPERTENSION



H O ♂ 60 MALIGNANT HYPERTENSION

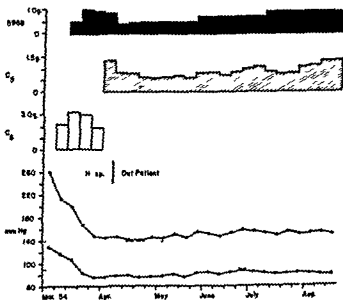


Fig 26

the full dose is given if the systolic pressure is over 140 mm half the dose if between 140 and 130 mm one fourth the dose if 130 and 120 mm and none if 120 or below Higher omit levels 10 mm apart are used in atherosclerotic and azotemic patients perhaps 150 140 and 130 or 160 150 and 140 depending upon where the diastolic pressure has settled or whether the azotemia has lessened or worsened Measurements are then made with the patient seated in order to take advantage of some postural hypo-

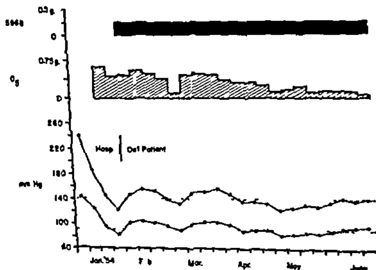
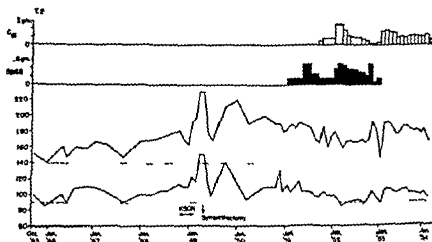


FIG 26 Examples of adequate control of blood pressure by giving adequate doses of ganglionic blocking agents (hatched area pentolinium bitartrate C_5) and hydralazine (solid black area 5968) In the case of H J in hospital control was poor at first until doses were raised sufficiently to achieve normotension. Each point represents the mean of six measurements Diastolic normotension was achieved. H O. Note increase in blocking agent required to maintain normotension in August. Hexamethonium chloride (C_6) was used at first in hospital. M B Note automatically decreasing doses when blocking drug was given on a sliding scale Each point is mean of 35



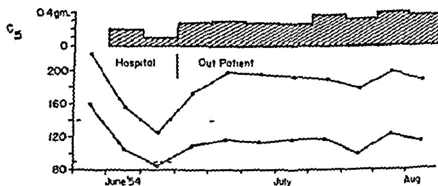


FIG 27 Examples of failure of control of hypertension by ganglionic blockade (pentolinium C_5) alone *E D* Although normotension was achieved in hospital patient died of malignant hypertension in May 1955 Hydralazine should have been added to achieve control *T P* Hydralazine (5968) was discontinued because of late toxicity Although malignant hypertension was relieved and did not recur ganglionic blockade with hexamethonium chloride (C_6) failed to maintain diastolic normotension High systolic pressures were necessary because of *postural hypertension resulting from drug* and surgical sympathectomy Each point is the mean of 150 measurements

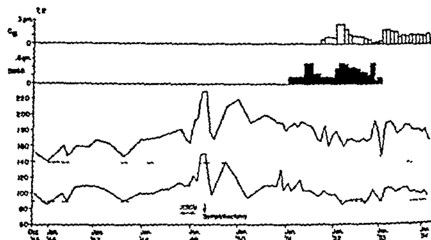
tension Standing pressures are avoided The night dose is omitted leaving eight hours of uninterrupted sleep

5 Parasympatholysis should be treated A nightly laxative and magnesium citrate if the bowels have not moved by mid morning will usually promote a daily evacuation It is important to prevent distension of the intestines (404)

Precautionary measures against obstruction of a hollow viscus already partially obstructed should be taken Abdominal scars prostatic hypertrophy, frequent rhinitis, are warning signs of possible trouble from this source They are usually less severe than the disease being treated When the drugs cannot be tolerated protoveratrine can be substituted with good results Surgical sympathectomy, of course does not carry this hazard Prostatic obstruction may require surgery

Use of Hydralazine This drug is given almost always in conjunction with either ganglionic blockade (Fig 28) or another milder agent acting on nerves (423) Because of initial side reactions mainly attributable to its antihistaminase action which are lessened with nerve acting drugs it is begun at doses of 25 mg every 4 hours raising the dose to 50 75 and 100 mg every 4 hours on 3 successive days It is given with the blocking agent Thus 500 mg a day is the usual dose in severe hypertension we have had to give as much as 1 0 Gm for short intervals These large doses may cause hydralazine disease in 10 per cent of patients after 6 months In general larger doses are given for greater nephrogenic components to the vasospasm smaller doses when the neurogenic component is large It is unreliable when used alone (189 396)

High fever aching and malaise appearing during the first few weeks of administration of hydralazine requires discontinuation or marked reduction of dosage Fortunately such sensitive individuals are rare Angina pectoris



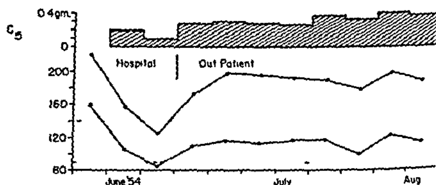


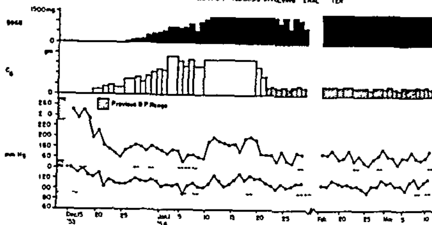
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can be made worse (or relieved) by hydralazine A compromise partial control of hypertension by neurogenically acting drugs plus perhaps restriction of dietary sodium may provide some measure of vascular safety which while not ideal may lengthen life In our experience patients with *severe* hypertension are rarely intolerant to this agent when it is first given

Hydralazine disease appearing after 6 to 24 months of ingestion of fairly large doses necessitates two courses Stopping the offending agent entirely results in return of hypertension in these patients the mortality rate from hypertensive causes is 10 per cent Large doses of ganglionic and hypothalamic blocking agents with or without protoveratrine usually fail to control the hypertension adequately wide swings from high to low levels take place daily This situation is about the most difficult to meet in therapy and we have no solution Low salt diets or thiocyanate might be used sodium azide has been valueless (Fig 29) Closely related analogues of hydralazine have caused recurrences of the disease and chemically less related ones have been relatively worthless

Hazards of severe restriction of dietary salt are well known The nephrosclerotic kidney is a salt losing kidney to some extent and hyponatremia with renal failure (the low salt syndrome) can be induced by limiting the intake to a point less than obligatory urinary losses Borderline renal function predisposes to this usually fatal condition (397 399) (Fig 30)

The second choice involves marked reduction of the dose and the possible addition of cortisone until symptoms subside The disease resembles in part a phenomenon of depletion By small doses blood pressure can be controlled although L E preparations may remain positive the disease remain in a subclinical lupoid stage and



A.K. ♂ 49 SEVERE BENIGN HYPERTENSION.

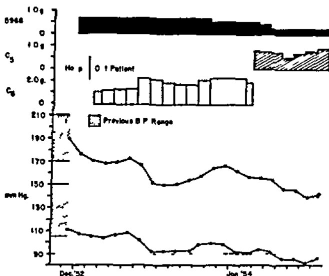


FIG 28 Example of effect of pentapyrrolidinium bitartrate (hatched area) as compared with that of hexamethonium chloride in malignant hypertension quite resistant to the latter Upper Curve The patient suffered from Leriche's syndrome proven by previous operation which probably extended a year later to involve the right renal artery as shown by contrast aortograms Each point is the average of six measurements made at four hour intervals during his hospital stay (Dec and Jan) and of five measurements as an out patient (a representative month Feb to March is shown) Large doses of hydralazine have been required While the result can be considered only fair the patient worked full time on a railroad section gang without symptoms and his grade IV ocular fundi cleared He died of uremia 2 years later Lower Curve Poor control of blood pressure in a patient taking adequate doses of hexamethonium chloride and insufficient doses of hydralazine after two years of good control Each point is the average of 35 measurements Pentapyrrolidinium bitartrate apparently altered a poor response into a good one Such is not always the case

creased the patient and his record are evaluated if rela tive normotension is not consistent and the fluctuations of pressure much less marked the dose of one or the other agent is adjusted upward until it is maintained Maximal (but unusual) single doses of hydralazine can be as high as 200 mg of hexamethonium chloride 1 0 Gm of pento-linium bitartrate 300 mg or more of chlorisondamine 100 mg and of mecamylamine 20 mg

Before discharge measurement of blood pressure with the patient seated causes the amount of ganglionic block

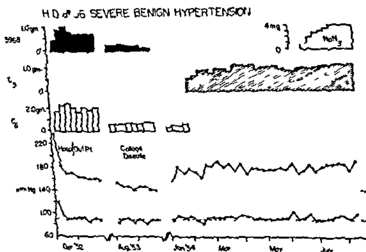
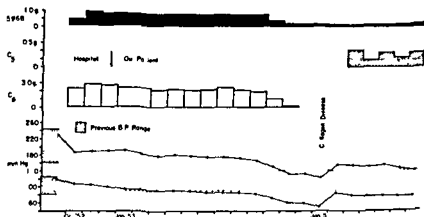


FIG 29 Examples of hydralazine (collagen) disease with special refer ence to blood pressure and difficulty of achieving control without hydralazine (See also *Fig 27*) *H D* Control of systolic normotension was impossible by ganglionic blockade when hydralazine (5968) was stopped. Sodium azide (NaN_3) had no demonstrable effect. J W Note strict normotension when hydralazine disease was once established. A sulfur containing pyridazine (13050) did not affect blood pressure nor did sodium azide W G represents the second choice that of continuing hydralazine in very much smaller doses. L-E cells appeared and disappeared several times in blood while this was done but normotension was maintained Each point represents the mean of 6 35 or 150 measurements depending upon the time scale

the patient s situation be potentially precarious Discovery of a substitute for hydralazine which will not cause this phenomenon is the only solution to the problem (Fig 29)

Combined Therapy with Ganglionic Blockade and Hydralazine Hydralazine is given regardless of the level of blood pressure when the two agents are used together It disappears rapidly from the blood At the end of the four day period during which the dose was progressively in



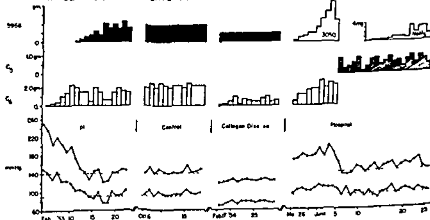


Fig 29

ing agent automatically to adjust itself as postural effects occur The usual result of omission of the night dose is a rise in the morning resting or basal pressure The patient is taught to take his own blood pressure five times a day before each dose and record it on special charts He is instructed on the actions of the drugs and their side effects He leaves the hospital on the same schedule which was designed to prevent both hypertension and hypotension By keeping a daily record trends can be observed which are invaluable for efficient therapy In our clinic we examine a patient one month after discharge from hospital and then at 3 to 6-month intervals if he is doing well Patients seldom complain of the inconvenience which takes about 15 minutes a day but they do object to the cost of the drugs

Treatment of Crises In hypertensive crises (pulmonary edema cerebral edema toxemia of pregnancy) requiring parenteral administration two lines 20 mm apart are drawn across the graphic chart at the level at which systolic pressure is to be maintained After initial lowering of blood pressure by a small dose of a blocking agent blood pressure is measured every hour and a subcutaneous injection of the full effective dose given if it is above the upper line half the dose if between the lines and none if below the bottom line Changes in total dosage must be made often The second day the two lines are drawn 20 mm lower Thus a patient with encephalopathy (wet brain) may have his pressure reduced from 300 mm Hg to 220 to 200 mm the first day 200 to 180 mm the second day and 180 to 160 mm the third day Usually oral medication becomes possible long before this time The pressure must be reduced more drastically when there is pulmonary edema We prefer parenteral ganglionic blocking agents to parenteral hydralazine because of their shorter

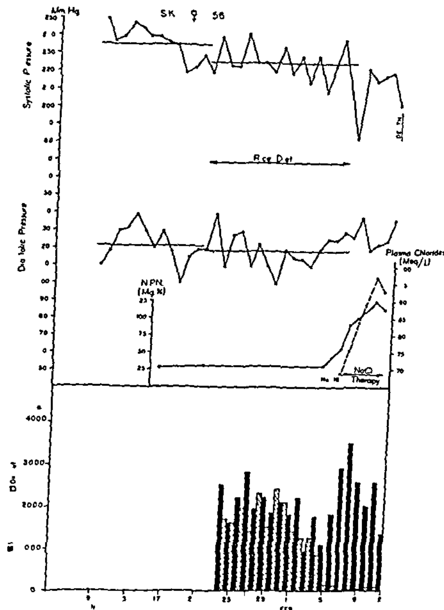


Fig 30 A woman aged *56* had rapidly progressive hypertensive vascular disease without retention of nitrogen but with diminution of renal function When she was given a diet containing 0 5 Gm of salt (rice diet) oliguria developed 14 days later This development was rapid and the patient complained of nervousness apathy loss of appetite and weakness Her intake of fluids remained high in spite of the obvious overhydration which was developing Plasma chlorides were low as was sodium and an attempt to reverse the oliguria by the use of intravenous hypertonic saline solution was to no avail She died of uremia (From Schroeder H A. *J.A.M.A*, *141* 117 1949)

FIG 31 Medication and vital signs during hospitalization of a 52 year-old white male with malignant hypertension and pre treatment azotemia The upper bar graph indicates the daily dose of autonomic blocking agent. The open bars represent oral pentolinium tartrate (po C_5) The cross hatched bars represent parenteral pentolinium tartrate (im C_5) and the solid bars represent parenteral hexamethonium chloride (im C_6) The potency of oral pentolinium tartrate approximates that of intramuscular hexamethonium chloride however the scale for intramuscular pentolinium tartrate has been expanded to allow for its greater effect The lower bar graph indicates oral hydralazine intake per day Daily blood pressure averages are shown each value being the mean of at least 6 and often as many as 24 readings taken with the patient in a sitting position The stippled area to the left indicates the pre treatment range of blood pressure The line graph at the bottom of the figure is a schematic representation of the vital signs The solid line shows the temperature in degrees centigrade which was below 37 5 except for three bouts of pyrexia The dotted line shows the pulse in beats per minute which was always below 80 except for two episodes of tachycardia. The figures indicate tachypnea with any daily average values above 30 breaths per minute being noted. The word *failure* denotes the three periods of cardiac decompensation Note the very high doses necessary once escape has occurred. Patient died (From Perry H M Jr O'Neal R M and Thomas W A, *Am J Med* in Press 1957)

actions with less tendency to produce prolonged hypotension In cerebral edema it may be necessary to avoid cerebral ischemia, as indicated by increasing coma by reducing cerebrospinal fluid pressure

Tolerance to the Action of Drugs No tolerance seems to develop when both types of drugs are used correctly and continuously On the other hand, tolerance is common when therapy is intermittent, an unexplained phenomenon In the larger sense it resembles bacterial resistance to antibiotics given in less than therapeutic amounts

One of the most difficult situations to meet is in the patient whose blood pressure has been lowered successfully, *only to have the drugs discontinued because of undue* alarm at side effects or the consequences of normotension (mental depression malaise, lassitude weakness) The hypertension which recurs immediately is much more resistant to therapy and requires much larger doses of drugs after a few days than it did initially, sometimes it seems impossible to treat (Fig 31) We have encountered no resistance in fresh untreated cases *the secret of successful therapy is continuous therapeutic pressure* While we cannot account for this phenomenon it is commonly observed and hazardous to the patient Many lives have been lost by nervous and eratic therapy (Fig 32)

Changes Occurring with Time If the dose of the ganglionic blocking agent automatically falls to negligible quantities (in 6 to 18 months) by reason of sustained normotension the dose of hydralazine is then reduced to four times a day, three times a day twice a day and finally halved at 2 to 6 month intervals Reduction in dosage is made very slowly (Fig 33) The amount of reserpine is halved in a month or two if drowsiness appears and then halved again until symptoms disappear (406)

What to Do If a Patient Is Not Doing Well The pa

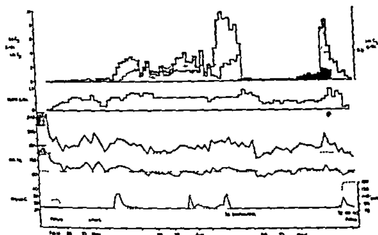


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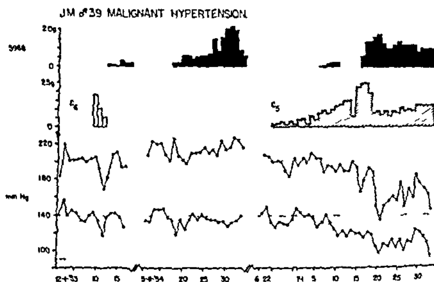


FIG 32 Example of logic of using both ganglionic blockade and hydralazine (5968) Neither drug was effective alone in large doses Hexamethonium (C_6) pentolinium (C_5) or hydralazine failed to alter course of malignant hypertension until combined therapy was begun when relative normotension was achieved These records were made in hospital and all readings are shown Obviously in this azotemic patient (NPN 76) with heart failure death would have been the outcome had only one drug been used or had both been given in inadequate doses

tients who do least well on combined therapy are usually men in the sixth decade with atherosclerosis, and not, as might be expected those in malignant stages Certain individuals, however because of a too early reduction in dosage or because of a dosage schedule sufficient for the hospital but insufficient for the stresses of active living continually show hypertensive levels reaching as high as 180 or even 200 mm Hg systolic during one of the five measurements a day Several choices are open a) The dose of one agent is increased for a month If not successful, the dose of the other agent is increased b) Protoveratrine in amounts in sufficient to cause nausea is begun c) Dietary salt is re

stricted moderately d) The patient is readmitted to the hospital and restabilized e) Pheochromocytoma is suspected in patients whose blood pressures fluctuate widely and cannot be controlled Reduction in dosage is not possible for those who keep themselves moderately hypertensive even after 3 years Those who do best are those who attain and maintain normotension (Fig 33) (406)

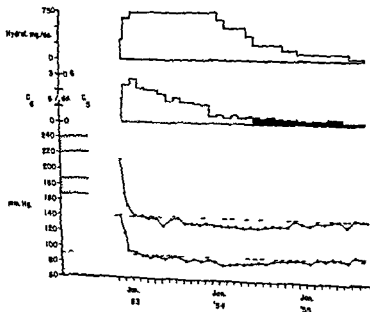


Fig 33 Blood pressure and oral medication for a 34 year-old white male with malignant stage of hypertension The range of pre treatment blood pressure is indicated by cross-hatching The bar graphs indicate the drug intake the open area representing hydralazine the dotted hexamethonium (C_6) and the solid pentolinium (C_5) Note that the scale is different for the two methonium compounds since the second is approximately five times more potent than the first. (From Perry H M Jr and Schroeder H A *Circulation* *13* 528 1956)

RESULTS EXPECTED

Since this monograph is not primarily concerned with a report on the results of therapy, only a brief resume of what can be expected with adequate therapy can be given The long term effects have been published (401-403 93 168 405 419) In general, those which could be predicted from an understanding of the disease occur, several un expected and unpredicted effects appeared as well

1 a) Heart failure due to left ventricular strain which accounts for over 50 per cent of the death rate is virtually

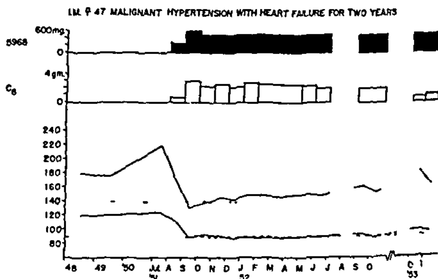


FIG 34 One of the first four patients to receive ganglionic blockade (*hexamethonium C_6) and hydralazine (5968)* Severe left and moderate right sided heart failure disappeared in three days grade IV fundi regressed digitalis was discontinued and salt was added to her diet (in November 1951) She subsequently became sloppy in her habits omitted her drugs from time to time began to complain of dyspnea and hemorrhages reappeared in the ocular fundi Hypertension was later controlled on larger doses of drugs and she remained symptom free From being invalided, she has been able to work full time in her own restaurant for five years as long as she keeps her blood pressure down

abolished this within a few days Salt can be added to the diet in from 2 to 6 months time Also in a few weeks digitalis can be eliminated (Fig 34) Only a rare individual continues to require digitalis and dietary restriction of salt We presume that these patients suffer from myocardial fibrosis due to atherosclerosis. Heart failure with only a moderate hypertension and much coronary arterial disease however is only moderately affected

1 b) Abnormal electrocardiographic patterns indicative of left ventricular strain revert to normal within several months Patterns suggestive of left ventricular hypertrophy revert to normal in some but not in all cases This may take 1 to 4 years Enlarged hearts often but not always become smaller in roentgenograms Time 1 to 5 years (406)

2 a) A few weeks after the start of therapy the progression of renal damage due to arteriolar nephrosclerosis is halted Unpredicted was a gradual return of depressed renal function in many but not all cases This occurs in from 1 to 4 years (405)

2 b) In from 1 to 6 weeks albuminuria diminishes or disappears When caused by pre-existing organic renal disease it remains at lessened quantities

2 c) In azotemic individuals nitrogen retention remains static or diminishes unless initial values are over about 60 mg per 100 ml of nonprotein nitrogen in the blood (Somogyi zinc precipitate corresponding to 75 to 90 mg per cent by the phosphotungstic acid precipitate method) Time weeks or months In those with higher values azotemia usually but not always progresses to uremia rarely however we have seen relatively acute elevations to 130 to 160 mg per cent return to much lower levels This may occur after 3 weeks or more (Fig 35 36)

2 d) Ocular fundi revert to normal Hemorrhages are

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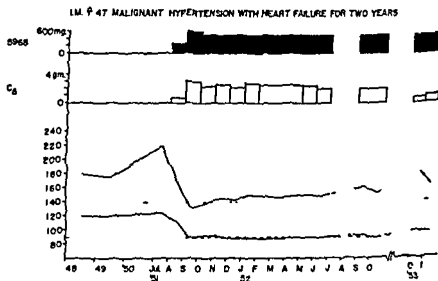


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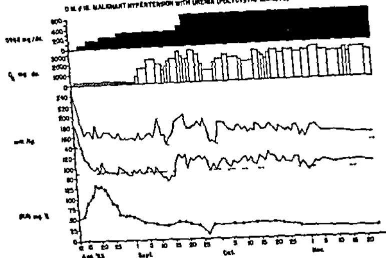


FIG 36 Medication blood pressure and nitrogen retention during hospitalization The solid bars represent oral hydralazine (5968) The cross hatched bars represent parenteral and the open bars oral hexamethonium chloride (C_6) The parenteral dose has been multiplied by 10 in order partially to compensate for the much greater efficacy of this route of administration Each of the points on the blood pressure curve is the average of at least six and initially as many as ?4 determinations All were made with the patient *supine* Note that azotemia is shown in terms of blood urea nitrogen rather than total nonprotein nitrogen

Except for life long enuresis this 18-year-old white male was entirely well until 3 days before he entered the hospital His mother had died with polycystic kidneys Pyrexia and malaise were the initial symptoms followed by lethargy emesis disorientation and coma. Physical examination revealed in addition papilledema hemorrhagic retinitis minimal cardiomegaly and a pre systolic gallop Roentgenologic examination suggested polycystic kidneys and 3 plus albuminuria was found After returning home the patient did very well He was working when last seen in July 19?5 at which time his physical examination including fundoscopic examination was normal His urine contained *no protein* His antihypertensive regimen called for a maximum dose of 750 mg *oral* hexamethonium chloride and a constant dose of 100 mg of hydralazine every *four* hours. His sitting blood pressure at home averaged 160/80 mm. Hg (From Perry H M Jr and Schroeder H A. *Circulation 14* 105 1956)

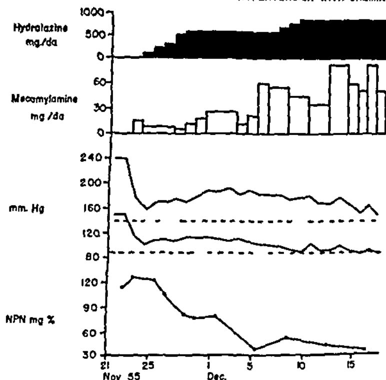


Fig 35 Medication blood pressure and nitrogen retention during hospitalization The solid bars represent oral hydralazine The open bars represent oral mecamylamine (Inversine Sharp & Dohme) Each of the points on blood pressure curve is the average of at least six and initially as many as 24 determinations All were made with the patient supine In addition to the medication shown 513 mg of intramuscular hexamethonium chloride were administered on November 22 without any effect on the blood pressure

Except for probable pyelonephritis 10 years previously followed by the discovery of high blood pressure this 45 year-old Negro policeman was essentially asymptomatic until 6 months before he entered Barnes Hospital His initial complaints were progressive asthenia and increasingly frequent periods of syncope associated with vertigo and amblyopia Urinary frequency and dyspnea appeared somewhat later finally nausea and vomiting became frequent There was hemorrhagic retinitis with bilateral papilledema and cardiomegaly Although cardiomegaly was present the lung fields were clear to percussion and auscultation and there was no edema There was cylindruria and 3 plus albuminuria only 100 ml of blood contained 10 gm of hemoglobin Both electrocardiograms and roentgenograms of the chest indicated left ventricular enlargement The decrease in azotemia and blood pressure following the institution of oral mecamylamine and hydralazine therapy is indicated in the graph This man was discharged from the hospital with a regular diet without digitalis and with no symptoms except amblyopia referable to his hypertension or to his therapy returning to work 3 weeks later (From Perry H M Jr and Schroeder H A. *Circulation 14* 105 1956)

absorbed in 2 to 6 weeks soft cotton wool exudates disappear in 1 to 4 weeks papilledema slowly regresses in 4 to 12 weeks and hard waxy exudates and scars shrink to nothing in 1 to 3 years

3 a) Atherosclerotic complications are less frequent In from 1 to 6 weeks angina pectoris usually disappears although rarely it becomes initially worse

3 b) The incidence of coronary occlusion appears somewhat lower (after 3 to 5 years) although this disease ac

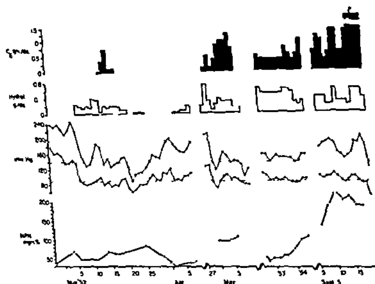


Fig 37 Two directions of progress in azotemia and malignant hypertension Upper The condition of G B a 54 year-old man has been static or improving slowly and he has been able to return to full occupation Control of blood pressure has been deliberately only fair in order to avoid further azotemia which has varied from 48 to 7? mg per cent NPN (mean 59) Middle That of M C has remained fairly static or improved slightly weight and health have increased. Lower That of L. T a 48 year-old man improved at first but azotemia later rapidly progressed to death. Pyelonephritis was found at autopsy the kidneys together weighed 105 Gm.

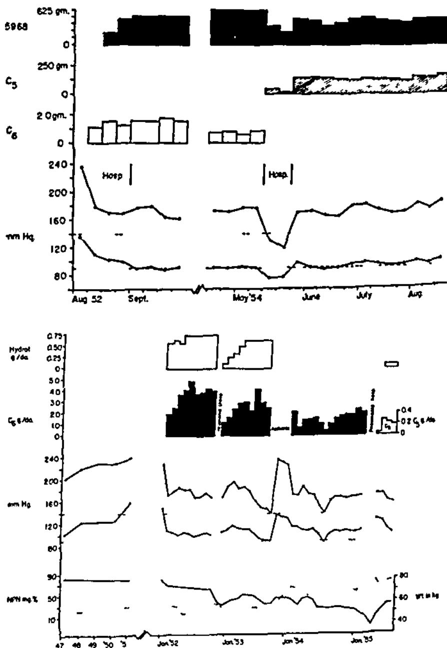

G.B. ♂ 52 MALIGNANT HYPERTENSION
625 gm.
5968
0
250 gm
C5
0
2.0 gm.
C6
0
240
Hosp
Hosp
200
160
mm Hg
120
80
Aug 52
Sept.
May '54
June
July
Aug.
Hydrol g/da.
0.75
0.50
0.25
0
C_6 g/da.
50
40
30
20
10
0
0.4
0.2 C_5 g/da.
0
240
200
mm Hg
160
120
80
90
NPN mg %
50
10
80
60 Wt in kg
40
47
48
49
50
51
Jan '52
Jan '53
Jan '54
Jan '55

FIG 37

absorbed in 2 to 6 weeks soft cotton wool exudates disappear in 1 to 4 weeks papilledema slowly regresses in 4 to 12 weeks and hard waxy exudates and scars shrink to nothing in 1 to 3 years

3 a) Atherosclerotic complications are less frequent In from 1 to 6 weeks angina pectoris usually disappears although rarely it becomes initially worse

3 b) The incidence of coronary occlusion appears somewhat lower (after 3 to 5 years) although this disease ac

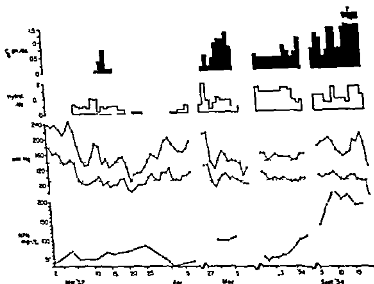


FIG 37 Two directions of progress in azotemia and malignant hypertension. Upper The condition of G B., a 54 year-old man has been static or improving slowly and he has been able to return to full occupation Control of blood pressure has been deliberately only fair in order to avoid further azotemia which has varied from 48 to 72 mg per cent NPN (mean 59) Middle That of M C. has remained fairly static or improved slightly weight and health have increased. Lower That of L. T a 48-year-old man improved at first but azotemia later rapidly progressed to death. Pyelonephritis was found at autopsy the kidneys together weighed 105 Gm.

counts for a major part of the mortality in treated patients

3 c) In 3 to 5 years the incidence of cerebral hemorrhage is markedly lessened in those patients who have suffered one attack

3 d) Also in 3 to 5 years the incidence of cerebral thrombosis is considerably lowered, but by no means abolished

4 a) In patients for whom treatment is considered mandatory, continuation causes increased life expectancy while discontinuation results in early death (Fig 37)

4 b) When a patient has survived 6 months of therapy, his chances of surviving 5 years are excellent

Surgery This monograph is not the place to discuss the pros and cons of surgical sympathectomy an operation which has definitely altered the outcome and prolonged the life of many patients (420, 421) In our experience drugs faithfully taken and properly administered have been of considerably greater value than surgical sympathectomy for the following reasons 1 Cases too far advanced for surgery and unoperable cases with azotemia can often be salvaged 2 All patients can be treated regardless of the stage or degree of vascular damage 3 Failures after surgical sympathectomy can be salvaged when hypertension has recurred and become severe 4 The mortality rates of the most severe cases is considerably less than those following operation

In actual practice no patient should be denied the choice of drugs or surgical operation when hypertension is doing harm When a patient is unwilling or unable to take drugs regularly surgery should be urged in suitable cases We must remember however that surgical sympathectomy even the subtotal variety can never block as many nerves as does adequate ganglionic blockade Therefore, in cases resistant to ganglionic blockade surgical intervention can be expected to fail contrariwise cases re

sponding well to ganglionic blockade alone can be expected to respond to surgery even though the usual lumbodorsal sympathectomy only removes 50 to 60 per cent of the nerves Operation therefore does not become the method of choice when medical measures fail for the opposite holds true i e drugs will work when surgery has failed The one advantage of surgical over chemical sympathectomy is the lack of bother to the patient when the result is successful

According to the data of White (421) when cardiovascular complications occur in hypertensive patients the mortality is high Left ventricular weakness and failure cerebrovascular accidents angina pectoris and myocardial infarctions cause a 3 year mortality rate of 82 per cent and a 10 year mortality rate of 96 per cent with a mean survival time of 4 1 years Surgical sympathectomy alters the 3 year rate to 24 per cent and the 10 year rate to 50 per cent with a mean survival time of 6 1 years for the deceased

TABLE L

MORTALITY RATES AT FOUR YEARS OF PATIENTS SUBJECTED TO SURGICAL SYMPATHECTOMY USUAL MEDICAL MEASURES AND CHEMOTHERAPY (PER CENT)

Smithwick Group	*Smithwick s Series (420) Av Ages 38–47*		*White s Series (421) Ages 30–60*		*Author s Series Ages 34–76 Chemotherapy*	
	Medical	*Surgical*	*Medical*	*Surgical*	*Stopped*	*Continued*
I	10	3			—	0
II	33	12			32	1
III	58	19	84	24	38	3
IV	87	52			100	20
Azotemia	(100)	†	(100)	†	100	45

* Most patients were 40–60

† Not suitable for operation because of high operative mortality

patients Chemotherapy properly maintained definitely decreases the mortality rate in 4 years to a point well below these figures (Table L)

Nephrectomy also can alter the course of hypertension when 1) unilateral renal disease is present 2) hypertension is early and not far advanced, and 3) the function of the opposite kidney is excellent When the hypertension has produced arteriolar nephrosclerosis in the good kidney removal of the offending one will naturally not result in 'cure Cases favorable for nephrectomy are rare *

Bilateral adrenalectomy is indicated only in cases exhibiting evidences of adrenal cortical hyperfunction Better diagnostic methods for overproduction of specific steroids may allow better selection for surgical therapy

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* *When any surgical operations are indicated* patients long treated by drugs will usually respond by strict normotension without drugs for one to two weeks after which hypertension will recur This phenomenon has also been seen after severe infections gastro-enteritis and trauma Apparently the ability of the peripheral vessels to respond to trauma is altered by these drugs for some days

Chapter IX

A PRELIMINARY APPROACH TO THE TREATMENT OF ATHEROSCLEROSIS

OBVIOUSLY, control of a patient s hypertension will do no more than relieve cardiac strain prevent further nephrosclerosis prevent cerebral hemorrhage and relieve angina pectoris Theoretically it will slow that part of the rate of progression of atherosclerosis which is dependant upon an elevated blood pressure Since atherosclerosis is probably reversible (443) at least in so far as cholesterol-containing plaques are concerned (and possibly calcification (422)) treatment of the whole patient and his diseases becomes essential for prolongation of a life potentially shortened by cardiovascular damage There fore an outline of the method we have used is given here the method involves practical measures based on theoreti cal approaches of most promise Since it is most difficult to measure alterations in this disorder for the better or worse until massive accidents occur only time will tell if the results are favorable

The serial measurement of lipoprotein fractions in blood is a procedure confined to the larger specialized centers Total plasma cholesterol however is readily measured in most hospital laboratories Based upon the assumption that lowered plasma cholesterol will in part prevent deposition of esters in plaques one can attempt to lower these values by using some of the influences dis cussed in Chapter VII

✓The method is based upon three influences 1) removal of some trace metals, 2) a diet low in animal fats but containing adequate vegetable fats, particularly linolenate, and 3) the provision of an adequate amount of pyridoxine For it is the possible effect of these biochemically and metabolically interrelated substances which can contribute to the disease (446)

METHOD

1 Diet As discussed in Chapter VII, the most important factor in the development of atherosclerosis probably lies

TABLE LI

LIST OF FOODS TO BE AVOIDED AS HAVING A HIGH SATURATED LOW UNSATURATED FATTY ACID CONTENT (348)

Food	*Reason for Avoidance*
Fats	
Coconut	*Short chain saturated*
Margarine	Hydrogenated vegetable oils
Palm	Short chain saturated
Cocoa	Short chain saturated
Hydrogenated vegetable oils and frying fats	Long chain saturated
Lard tallow	*Long chain saturated*
Butter cream cheese whole milk	Short chain saturated
Hydrogenated peanut butter	Long chain saturated
Proteins	
Pork and pork products	Mainly saturated
Fat meats*	Mainly saturated
Sweetbreads	*Not investigated*
Domestic goose and duck	Fattened not investigated
Processed meats of all kinds	Contain much fat
Hamburgers	Contain much fat

NOTE The Pure Food and Drug Administration requires that all processed foods be labeled correctly The labels should be read and those foods containing 'hydrogenated shortening hydrogenated vegetable shortenings or pure meat products should be avoided

* The fat meats are beef rib roast corned beef and tongue lamb loin and shoulder and mutton

in the kind of fat in the American and European diet From 30 to 40 per cent of the caloric intake comes from fat mainly of animal origin The purpose of the diet therefore is to restrict animal fat and hydrogenated vegetable oils and to provide an adequate intake of unsaturated vegetable fats containing linolenic acid The basic rules are

A No obvious fat of animal origin should be eaten Modern methods of fattening cattle for slaughter make a saturated body fat

B No hydrogenated vegetable oils should be used since hydrogenation saturates an unsaturated fatty acid (Table LI)

C Natural fat of vegetable origin containing the higher unsaturated fatty acids can be eaten in amounts as large as practicable since these contain the essential unsaturated fatty acids linolenic and linoleic

D In general reduce the fat content of the diet to about 20 per cent of the caloric intake

The most available sources of essential fatty acids are in soy bean and corn oil with the following iodine numbers

	Iodine No	*Remarks*
Soy bean oil	130	Contains 11% linolenate
Corn oil	115	Contains 0.5% linolenate
Cottonseed oil	105	Contains no linolenate
Sesame oil	103	Contains no linolenate
Peanut oil	85	Atherogenic in animals

In order to obtain enough protein without animal fats the following are recommended

All kinds of fish and shellfish Fish oils have a high iodine number

Poultry and game avoiding the fat (except domestic goose and duck) Chicken fat is high in linoleate

Lean beef lamb and veal Most animal fat is low in

linoleate and linolenate There is fat in muscle fibres of cattle force fed on corn before slaughtering

Legumes, such as peas, beans, lima beans soy beans and its products Their fats are largely unsaturated

Skim milk and fat free buttermilk Butter cheese, cream and whole milk contain principally short-chain saturated fatty acids, butter raises plasma cholesterol

Cereals

Some breads use hydrogenated vegetable oils for shortening

Eggs Yolks are fatty Fry or scramble in soy bean oil

Meat soups only if all fat is skimmed off at icebox temperature

Most canned soups contain butter, cream or fat

Salad dressings made of soy bean oil

All nuts, especially walnuts Seed oils contain unsaturated fatty acids

Curd cottage cheese and other fat free cheeses

The two vegetable oils should be used for shortening

Deep frying should be done only in corn or preferably soy bean oil

II Vitamins The second point of attack lies in the daily use of pyridoxine or pyridoxal, deficiency of which has caused the early lesions of atherosclerosis in animals and which is low in many processed foods About 5 to 10 mg per day is more than adequate

III Trace Metals Excessive amounts of certain trace metals in American human tissues may be concerned in cholesterol formation or the metabolism of fats One tablet, 0 5 Gm , of Calcium Versenate (EDTA) twice a day, or another similar compound, may chelate and remove these metals In some people, this substance alone will lower the cholesterol level in blood (Table LII)

Calcium If calcium deposits are demonstrable in blood

TABLE LII

CHANGE IN PLASMA CHOLESTEROL WITH ORAL EDTA (1 0 GM /DAY)

Patients	Sex	Age	Control (mg %)	Change (mg %)	Interval (weeks)	Major Diagnosis
W H.	♂	54	293	−134	20	Angina pectoris
B McD	♂	54	278	−150	35	Peripheral vascular disease
I S.	♀	70	276	− 40	4	Arterial hypertension
E. B.	♂	62	233	− 58	16	Coronary occlusion, convalescent
G H	♂	45	252	− 36	25	Angina pectoris
G S.	♀	49	237	− 37	14	Arterial hypertension
E. S.	♂	77	225	− 74	3	Arterial hypertension
E. S.	♂	48	210	+ 18	44	Angina pectoris
H D	♂	63	189	− 49	8	A gina pectoris
R. S.	♂	54	177	− 38	12	Angina pectoris
J B	♀	59	178	+ 16	4	Arterial hypertension
Mea			233	− 55		

vessels and symptoms or signs are present the method of Clarke Clarke and Mosher for removing metastatic calcium may be used (422) Trisodium EDTA, 5 0 Gm in 500 ml 5 per cent glucose solution is slowly infused intravenously over 2 to 6 hours The patient is taught to slow the infusion at the appearance of unusual symptoms Strangely enough hypocalcemic tetany does not appear under these precautions Ionized calcium salts and calcium chelated to proteins and peptides at a weaker stability constant than 10 6 (Log K_2 EDTA) are probably removed the strongly chelated calcium in bone is probably not

An injection is given daily for 5 days 2 days are allowed for rest and the 5-day course repeated After a month or more for evaluation of symptoms a second 50 Gm is administered

RESULTS EXPECTED

In Table LII are shown changes in blood cholesterol levels using calcium disodium ethylenediamine tetra acetate (Calcium Versenate) in doses of 1 0 Gm per day In Table LIII are shown the changes produced by this agent

TABLE LIII

Effect of Regimen on Plasma Cholesterol (mg %)

Patient	Age Sex	Pre treatment Range		Pre treatment Mean	No Samples	2 Mo	4 Mo	Range After 3 Mo	No Samples	Diagnosis and Remarks
H Sch.	49 ♂	237	244	240	2	146	168	146–172	12	Normal
E St.	70 ♀	276	278	277	2	151	216	216–227	12	Atherosclerosis
C Cru	62 ♂	260	300	280	3	236	200	112–237	9	Hypertension
S Mat	61 ♀	207	240	224	2	146	198	190–234	6	Hypertension
K. Sch	59 ♂	200	217	212	4	120	141	117–154	8	Hypertension
G Nes.	45 ♂	213	234	223	4	178	180	159–197	7	Hypertension
D Blu	49 ♂	219	295	244	4	179	204	176–204	6	Hypertension
G Boa	55 ♂	233	272	261	4	155	137	137–272	6	Hypertension and Atherosclerosis
B McD	52 ♂	204	278	241	2	145	128	105–156	8	Peripheral Vascular Disease
C Gri	57 ♂	210	253	234	3	206	181	171–201	6	Hypertension and Leriche s Syndrome
A Han	60 ♀	174	207	194	3	168	171	171–200	5	Mild Hypertension
E Fri.	69 ♀	217	231	224	2	128	185	161–196	3	Hypertension
E Shr	49 ♂	228	249	239	3	119	232	119–232	4	Angina Pectoris
M Bro	72 ♀	245	276	257	4	237	172	172 184	3	Atherosclerosis
L Bat	62 ♀	256	300	280	3	183	250	241–250	7	Atherosclerosis
R. She	55 ♂	162	176	170	5	121	131	114–140	8	Mild Hypertension
D Lik.	56 ♀	481	624	552	6	377	366	366–422	3	Xanthomatosis
A Bla.	48 ♂	162	259	209	4	216	287		4	Hypertension
G Hir	46 ♂	238	262	250	3	185			1	Angina Pectoris
R Ber	53 ♀	299	323	312	2	279	298	298–321	4	Hypercholesterolemia
Mean				258		181	200			

with diet and vitamin B_6 added In general the cholesterol changes are downward although in some cases they are resistant to all three forms of therapy Some depressed values did not rise when EDTA was discontinued an expected result if trace metals were being removed

All patients with angina pectoris were relieved of attacks of pain either completely or partly in that they occurred less than once a month No electrocardiographic changes in the direction of normal were observed No signs of hepatocellular damage developed

Comment While untried for periods long enough to evaluate these results on the disease there is little doubt that cholesterol values can become quite low by this form of treatment Changes in the degree of atherosclerosis are difficult to measure but rough estimates of improvement in the disease can be estimated especially when it has advanced far enough to give local ischemic symptoms In the coronary arteries relief of angina pectoris if it is real and not imaginary suggests resorption of plaques In the aorta lessening of the widened pulse pressure suggests a return of aortic elasticity In the legs relief of claudication indicates improvement in blood flow In the cerebral area abolition of minor paraesthesias and paralytic episodes indicates reabsorption of plaques Some changes may be expected in time except for a return of aortic elasticity

Therefore if degenerative cardiovascular disease is to be treated as many predisposing parameters as possible must be altered favorably Today these lie in blood pressure the health of vascular intima and the lipid esters of cholesterol

Chapter X

SUMMARY AND INTERPRETATIONS

NATURE is not prodigal with biologic functions other than those for reproduction Metabolic processes may have one or two alternate routes but Nature does not provide dozens of methods by which defenses of a single function are maintained, by which digestion and combustion of a single substance proceeds, or by which homeostasis is maintained When an alternate pathway such as an anaerobic one, is substituted for an oxidation, malignant growth may result It is proper, therefore to look at the many and complex mechanisms which Man in his erratic searchings has partly uncovered and try to unify them toward simplicity in an imitation of Nature To do this we must depend upon evidence, in part factual and in part theoretical, delving into chemical causes

Psychosomatic While the psyche may affect the soma, the reverse is also true Realization that some areas of cerebral function may depend upon the valence of nitrogen in certain configurations, that one primary amine mediates one area of the brain and another can affect another and that the anatomic chemistry of phospholipids may influence healthy function, has opened up a wide field of investigation into the causes of mental derangements

The psychic manifestations of arterial hypertension, when not due to organic cerebral vascular disease, are best explained by the effects of primary amines produced by intermittently or permanently ischemic kidneys These

manifestations include emotional tension anxiety, excessive drive nervousness and the diencephalic blush The blush is induced by histamine and resembles that seen with excessive quantities of circulating serotonin tension nervousness anxiety result in some individuals from epinephrine isoamylamine tyramine and those synthetic or natural methylated analogues which inhibit cerebral monamine oxidase (ephedrine amphetamine etc.) thus preventing oxidative deamination of naturally occurring substances

From the huge amounts of tranquillizers sold the American public one might believe that chemically mediated nervous disorders were almost a national disorder That many individuals might be so affected could be inferred from the abnormal trace metal content of American tissues if one interfered with vanadium or monamine oxidase or there was deficiency of vanadium primary amines could be implicated as causes of a widespread cerebral disorder

Hereditary The ability to react to stress by vasospasm is an hereditary trait apparently transmitted as a Mendelian dominant

Neurogenic The sympathetic nervous system is over active most likely because of increased cortico-hypothalamic activity The posterior hypothalamus for which serotonin has a predilection is apparently stimulated more than is the anterior the chemical mediator of which is not known Cortico-hypothalamic activity is increased as a result of somatically formed primary amines Neurogenic vasospasm causes neurogenic renal ischemia

Renal Renal disturbances dependent upon ischemia produce humoral vasoconstrictor substances Trace metals both normal and abnormal are involved Two metabolic pathways may be considered

I Anatomic causes of ischemia usually depend upon intrarenal parenchymal disease, atherosclerotic narrowing of renal arteries, or arterial and arteriolar nephrosclerosis secondary to hypertension When the ability to react to stress by vasospasm is combined in one individual with organic renal ischemia, hypertension becomes permanent The first two renal disorders are anatomic accidents the last is caused by hypertension

Anatomic or intermittent functional ischemia produces enzymatic disturbances in the kidney The expected biochemical alterations resulting are

A Reduction of oxidative deamination of amino acids capable of anaerobic decarboxylation The results in the kidney

a) Less urinary ammonia formed per mol of bicarbonate (theoretical) Ammonia from glutamine however would continue to be formed anaerobically

b) A change of pH in the cortex to the acid direction (found)

c) Substitution of sodium for ammonia in order to maintain acid base balance in tubule (theoretical but logically inferred)

The urinary results

a) Urinary NH_3/acid ratio reduced (found)

b) Acid urine (usually found)

c) Sodium loss (found)

The expected remote results

a) More primary amines in blood (found)

b) Release of renin (found in acute states)

c) Stimulation of the adrenal cortex to production of aldosterone in an attempt to prevent excessive sodium loss (found) Animals (and human beings) might therefore eat a little more salt in order to compensate (found in rats)

B Renin is released possibly because of the acidity secondary to the lessened formation of ammonia (renin is extracted from kidney only at acid pH) This postulate is unproven and not too sound but we have no better one Renin comes from the superficial areas of the cortex of the kidney which becomes markedly acid when the renal artery is constricted Adjustments take place with time—several weeks

Result

a) Hypertensin (angiotonin) is formed in blood at first through the physical release of renin (found)

b) With time renin itself is no longer released into blood but continues to act *in situ* (not proven but renin disappears from blood) Perhaps renin is slowly modified into a somewhat different proteolytic enzyme

c) Hypertensin I or its analogue formed in kidney in active on blood vessels becomes activated either 1) through decarboxylation leaving an active terminal NH_2 the decarboxylase being in blood and kidney or 2) through action of a specific peptidase splitting off one or two amino acids and leaving a terminal $-NH_2$ In this latter event the peptidase would necessarily be a manganous enzyme The second pathway is the more logical one as peptidases are known and peptide decarboxylases are not Ordinarily in the absence of renal ischemia the small amounts of renin released into the renal venous blood form hypertensin which is inactivated both in kidney and in blood In renal ischemia the shift of locus of catabolism of hypertensin is from kidney to peripheral vasculature (theoretical but monamine oxidase acts on both hypertensin and pherentasin both peptides and it probably occurs in smooth muscle of blood vessels)

d) Pherentasin is actually human hypertensin II, the amino acid content of which could be expected to differ from that obtained from bovine, horse, or hog globulin (unproven but very likely)

III Enzymatic disturbances somewhat different from those resulting from organic renal ischemia may be caused by the accumulation of abnormal trace metals, notably cadmium Cadmium is a nephrotoxic substance Mercury and cadmium are the only metals which can readily displace zinc on a specific chelate being in the same periodic group, having the same coordination number and making the same shaped complex The expected results on renal *acid base equilibrium*

A Inhibition of carbonic anhydrase by displacement of zinc (probable but not proven)
   1 More acid in urine
   2 More base needed to neutralize acid i e ammonia or sodium

B Inhibition of decarboxylases by displacement of zinc *on pyridoxal enzymes causing a local vitamin* B_6 deficiency (one decarboxylase known to be inhibited Zinc displacement logical but unproven)
   1 Less decarboxylation of amino acids other than glutamine
   2 Less primary amines formed
   3 Less ammonia available for urine
   4 *Sodium wastage*

C Inhibition of transaminase a pyridoxal enzyme which probably contains a metal (unproven but possible)
   1 Less transamination from glycine, aspartic, glutamic and other amino acids as a source of urinary ammonia (transamination not proven to be a source of urinary ammonia)
   2 *Sodium wastage*

D Aminoaciduria (found) either because of process B or interference with tubular reabsorption by inhibition of a carrier metalloenzyme probably containing zinc

The net results would be cortical acidity less urinary ammonia and sodium wastage the same as those found in organic renal ischemia but appearing by somewhat different routes Primary amines in blood, however would not be elevated (Fig 38)

(If cortical acidity is the stimulus for the action of renin hypertensin I would be formed *in situ* and released into renal venous blood where it would be converted to hypertensin II (pherentasin) by action of a specific manganous peptidase Thus both organic renal ischemia and cadmium can cause the same end results Naturally renal ischemia accompanying neurogenic or pherentasin vasoconstriction would call into action pathway I

Therefore trace metals can be involved in the hypothetical peptidase probably manganous which converts hypertensin I to hypertensin II in monamine oxidase which inactivates it in the decarboxylases and amine oxidases which are concerned in the hypertensive state and even in tyrosinase (copper) which can inactivate norepinephrine epinephrine hydroxytyramine and tyramine)

Therapeutic Note Drugs or procedures which block sympathetic nerve impulses will counteract only the neurogenic portion of hypertension Drugs or procedures which a) act to dilate vascular smooth muscle b) increase renal plasma flow or c) inactivate hypertensin II or pherentasin will counteract the nephrogenic portion of hypertension All known inactivators are metal binding or chelating agents

The actions of hydralazine are fourfold

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  1 Less transamination from glycine aspartic, glutamic and other amino acids as a source of urinary ammonia (transamination not proven to be a source of urinary ammonia)
  2 Sodium wastage

1 Decarboxylase inhibition with the result that renal amino acid metabolism is further suppressed and therefore less primary amine is formed

2 Monamine oxidase stimulation with the result that those primary amines which are formed are oxidized more readily These include pherentasin or hypertensin II

3 Hypertensin and pherentasin are both directly in activated either through carbonyl linkage or what is more probable removal of a chelated trace metal necessary for the integrity of the peptides

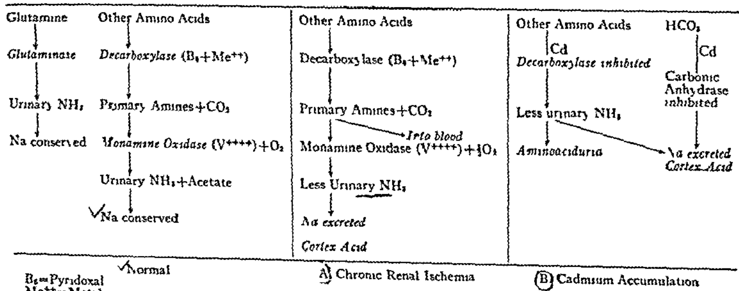
4 *Constricted* vascular smooth muscle is dilated no matter what causes the constriction by some unknown process which could be dependent either upon carbonyl or sulfhydryl binding or upon metal chelation

In addition histaminase is inhibited a reaction common to many hydrazides

Adrenocortical Renal sodium wastage (or need as in heart failure) probably causes adrenal cortical production of aldosterone (theoretical but logical) This steroid probably sensitizes blood vessels to circulating vasoactive amines and sympathetic discharges through intracellular sodium potassium alterations (proven only for DOCA) Most cases of hypertension exhibit secondary aldosteronism because of renal sodium wastage

Primary aldosteronism by sensitizing vascular smooth muscle to normally circulating vasoactive amines and normal sympathetic tone can produce a moderate degree of hypertension with normal renal plasma flow Cases of this nature are not unusual This type of hypertension while benign can slowly develop into a more serious variety with congestive heart failure the usual end result

Therapeutic Note While dietary salt restriction may induce enough sodium loss to negate the sensitizing effect of cortical steroids and salt on blood vessels it stimulates



B_6=Pyridoxal
Me^{++}=Metal
The reactions in italics have been demonstrated Transamination to α ketoglutarate from other amino acids has not been shown for the sake of simplicity

FIG 38 Proposed intermediary metabolism in ischemic and cadmium loaded kidney

(2) Cholesterol has a predilection for making unsaturated fatty acid esters When insufficient unsaturated fatty acids are available for esterification saturated fatty acids are used These esters are quite insoluble and probably have lower specific gravities Possibly breakdown metabolism or excretion of cholesterol is more easily accomplished when esters are unsaturated than when made of saturated long chain fatty acids

These two ideas are highly speculative The mechanism of lowering plasma cholesterol by essential fatty acids is not understood

Factors which may influence the deposition of cholesterol esters in sub-intimal spaces are

C *Physical*—Intra arterial pressure and changes of pressure (turbulence) at bifurcations of major vessels (Found)

D *Metabolic*—Pyridoxal deficiency causes sub-intimal lesions identical microscopically to pre atherosclerotic lesions observed in animals and man (Found)

a) Pyridoxal is necessary for the integrity of the mucopolysaccharides of sub-intimal ground substance (Inferred)

b) Pyridoxal affects fatty acid metabolism by promoting the synthesis of essential fatty acids from less unsaturated ones (Found)

c) Experimental pyridoxal deficiency and essential acid deficiency are quite similar in signs differing only in a few basic enzymatic disturbances (Found) Vitamin B_6 will partly relieve essential fatty acid deficiency essential fatty acids will partly relieve vitamin B_6 deficiency

The biochemical interrelationships of three of these influences are

1 Pyridoxal usually requires a metal for enzymatic activity One abnormal metal (cadmium) can inhibit a pyridoxal metalloenzyme

the adrenal zona glomerulosa to overactivity The result secondary hyperaldosteronism with salt depletion

Atherosclerosis Treated hypertensive patients no longer die of heart failure or renal failure when treated soon enough They die of the effects of atherosclerosis In order to prolong life, both blood pressure and blood lipids must be reduced

Factors which may affect cholesterol synthesis or de gradation and therefore atherosclerosis are -

A *Trace Metals* 1 Chromium increases hepatic syn thesis (in rats) Vanadium depresses hepatic synthesis Manganese may be the normal metallic mediator of synthesis

2 EDTA lowers cholesterol levels in man moderately or markedly, almost surely by chelation and removal of a metal from an hepatic enzyme concerned in synthesis or catabolism

B *Essential Fatty Acids* Fats containing linolenic acid (and possibly arachidonic acid) lower plasma cholesterol in man even when given in excessive quantities Fats not containing linolenic acid raise plasma cholesterol The mechanisms are not known but two can be hypothecated

(1) Squalene is a probable precursor of cholesterol Squalene ($C_{30}H_{50}$) can be considered as a highly unsatu rated C_{24} hydrocarbon chain with 6 extra methyl side groups and double bonds at the 2, 6 10, 14 18 and 22 positions or 4 carbon atoms apart Linolenic acid (C_{18}) has double bonds at 9, 12 15 positions and arachidonic acid at 5 8 11, 14 positions or 3 carbon atoms apart It would be impossible to use any combination of ethylene groups from these two acids in the structure of squalene Degrada tion to acetate and subsequent synthesis would be required No one knows much about this matter, except that squalene markedly accelerates synthesis of cholesterol

application of therapy present and to come Rapid im provements are expected

(1) Reduce blood pressure of hypertensive patients to a mean level of 140/90 mm Hg (or as low as tolerable) and keep it there indefinitely Two drugs are usually necessary given frequently regularly and carefully one should act on nerves and the other on vascular smooth muscle

(2) Lower plasma cholesterol to 120 to 160 mg per 100 ml (or about the same levels in mg per cent as systolic pressure is in mm Hg!) This can be accomplished slowly in some individuals and soon will be in most by

a) Metal chelation probably removing from liver an abnormal trace metal affecting synthesis Chelation and re moval of metastatic calcium in blood vessels can probably be accomplished when desirable

b) [illegible]et based on Less total fat to about 20 per cent of [illegible] intake Less animal fat especially saturated fatty [illegible]eral dairy products and pork are avoided A[illegible]lenic acid (probably 0 5 Gm per day is enoug[illegible]leic and arachidonic

c) Enough pyridoxine or pyridoxal Probably 5 mg per day is more than adequate This coenzyme is given for logical but untested reasons

It is a long step from a lowered cholesterol to absorp tion of plaques but the assumption is reasonable

There are enough ideas now under experimental ob servation to strengthen the belief that atherosclerosis is a reversible disease at least in so far as the fatty and calcific deposits are concerned Arterial hypertension in man can be controlled indefinitely and sometimes reversed Ap plication of therapy to both diseases in the same individual may be expected to reverse in part the lethal and disabling effects of each

2 Pyridoxal affects unsaturated acid synthesis by promoting further desaturation to essential fatty acids (linoleic to arachidonic linolenic to hexaenoic)

3 Pyridoxal lowers plasma cholesterol in monkeys deficient of this coenzyme and fed cholesterol ✓

4 Metals affect synthesis of cholesterol and fatty acids

5 Removal of unknown metals lowers plasma cholesterol in man

6 Feeding essential fatty acids lowers plasma cholesterol in man

An hypothetical schema to include these influences is seen in Figure 39

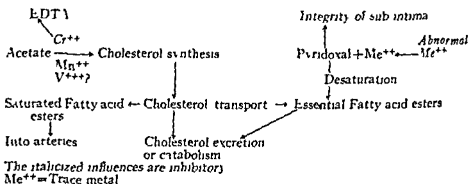


FIG 39 Hypothetical interactions of metals essential fatty acids and pyridoxal

Therapeutic Note Thus it would appear that therapeutic tools are now available to control or treat cardiovascular renal diseases quite specifically While these tools represent first approximations, they are powerful enough and practical enough to be effective in any individual who wants to be treated and is willing to give the time and energy to do so Since cardiovascular renal diseases account for over half the American death rate, *considerable* increase in our national longevity can be expected from wide

application of therapy present and to come Rapid im provements are expected

(1) Reduce blood pressure of hypertensive patients to a mean level of 140/90 mm Hg (or as low as tolerable) and keep it there indefinitely Two drugs are usually necessary given frequently regularly and carefully one should act on nerves and the other on vascular smooth muscle

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This book has attempted to give an orientation from biochemical abnormalities to clinical findings and specific therapy as the only satisfactory way to explain a disease and its control As usual in scientific medicine, much more needs to be known than is known but the directions for research are clear

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groups of patients as regards the clinical course the prognosis the survival rate and the incidence of coronary vascular accidents

SCHETTLER Concerning Dr HOOBLER's question on the heparin treatment of hypercholesterolemia I think that heparin has its indication only in cases with essential hyperlipaemia and in cases with very massive and very severe disturbances of fat metabolism We could not find any good effects in cases with hypercholesteronaemia and with those of a *familial* type I think this is very important to know We administered 100 mg as a depot for one week over a period of one year When not using a depot preparation we administered twice a week between 100 and 200 mg of heparin which is below the effective dose for coagulation But if I may repeat we only saw good results in this small number of cases with essential hyperlipaemia

With regard to the question of Dr HOOD we intend to reduce our diets from 3000 calories to 2300 and we give higher caloric diets only for persons who are working hard If we apply diets with 2300 calories we allow about 40 g (cc) of oil 20 g of fats and 20 to 30 g of hidden fats in the food It is worthwhile to reduce diets and to reduce weight in patients with coronary attacks

To Dr COTTET We do not know very much about the metabolism of high unsaturated fatty acids in correspondance to the saturated ones I do not believe that there is a strict antagonism between these acids According to American investigators it is correct that the unsaturated fatty acids do enhance the excretion of bile acids but one does not know in which way these substances are affected And I think it is impossible to use a diet — your second question — which contains only unsaturated fatty acids It is impossible at least in Western Europe Nobody will eat it and I am sure in America it is the same There are only small groups of phrenetic dietetic sects who use these diets

Now the third point the prognostic value of cholesterol and lipoproteins I think that the diagnostic value of lipoproteins and cholesterol in cardiovascular disease in coronary disease is very slight but not their pathogenetic significance I think it is true that high values of lipoproteins and cholesterol are harmful to the vessels

COTTET I agree with you

Closing remarks

By

F C Bieri

Gentlemen now that our meeting draws to its close allow me first of all to say how much I have appreciated your kind collaboration The pleasant atmosphere of this symposium which each of you has helped personally to create has I feel been particularly conducive to a free and frank exchange of views Speaking for myself I have derived a great deal from these debates of ours and my reaction has been one of both humility and admiration humility in face of the immense and complex problem with which we have been dealing and admiration for the splendid work which is everywhere being undertaken with faith conscientiousness determination and critical judgment

While taking part in these discussions over the past few days I have been struck once again by the number of pitfalls awaiting us whenever we try to tackle a problem as vast as that of hypertension Since no doubt all of you at some time or other will have fallen into one of these traps perhaps you will permit me to mention a few

To begin with there are the difficulties of method These arise for example when we endeavour to determine adrenal hormone concentrations in the urine or in the blood The discussion which followed the papers by Dr GENEST and Dr SCHWARTZ struck me as being very instructive in this connection The same reservations have to be made when analysing renin or hypertensin It would be a good thing if in future when confronted with diverging results authors were to carefully compare their techniques and try to discover why their findings do not tally before proceeding to interpret their results

Another pitfall consists in seeing only one detailed facet of a problem without considering it in relation to the whole Modern investigations are of such complexity that one is often compelled to concentrate on one particular aspect of a question Where the results obtained prove interesting one is tempted to exaggerate their intrinsic importance instead of trying to integrate them into the overall picture

Often it is difficult to determine the causal connections between two phenomena observed at one and the same time as for example in the case of hyperaldosteronuria and hypertension or arteriolar necrosis and malignant hypertension That the problem of arteriolar necrosis now seems to have been partly solved is due to the fact that it is possible to reproduce this lesion in animals by raising their blood pressure and to prevent its development in hypertensive patients by means of anti hypertensive therapy On the other hand there is nothing to prove that essential hypertension is due to hyperaldosteronism In the absence of experimental proof and clinical arguments, it might equally well be supposed that the hyperaldosteronuria is secondary to the rise in blood pressure

I am quite convinced of the importance of experimental proofs in arriving at a correct interpretation of a given set of clinical facts but it should not be forgotten that phenomena observed or provoked in the rat the rabbit or the dog cannot always be translated *to human pathology* Dr GROSS *has rightly emphasised how easy* it is to produce hypertension in rats On the other hand we all know how comparatively unsusceptible dogs are to arteriosclerosis Thus the fact that a hypertensive dog shows very little tendency to nephro angiosclerosis does not mean to say that in man the vascular lesions encountered are not partly due to elevated blood pressure Again the fact that rats treated with salt and corticosteroids develop hypertension and nephrosclerosis is no proof that essential hypertension in man is caused by an adrenal disorder

However tempting it is wrong to interpret a chronic metabolic or haemodynamic upset on the basis of short term experiments An experiment by its very nature is of limited duration Now the effects of a drug may be quite different depending on whether the results are determined immediately after a single intravenous injection or after months of continuous treatment We have seen this in connection with the action of hypotensive agents on renal function and during the discussion following the paper by Dr FREIS Though it is true that chlorothiazide begins by reducing the cardiac output it ultimately leads to a decrease in peripheral resistance

Finally another grave danger to be guarded against is that of considering an hypothesis as an established fact Science would hardly be conceivable without hypotheses Every research worker has to fall back on them once he is in possession of a certain number of experimental findings since only by forming an hypothesis can he obtain some idea as to the logical direction in which concentrate his efforts Nevertheless every hypothesis must be abandoned as soon as it no longer tallies with the objective findings The survival

of a mistaken hypothesis constitutes a severe handicap to scientific research As for faulty hypotheses that have been set up as dogmas suffice it to say that they doom all effort to frustration

These few remarks of mine are so obvious that they no doubt strike you as superfluous I should now like to turn to a few points which the past few days discussions have perhaps helped to clarify

I was particularly pleased that Dr PICKERING and Dr PLATT had an opportunity of giving us their points of view Without wishing to repeat the arguments you have already heard I have the impression that in the end there was some measure of reconciliation between diverging conceptions and that at any rate several misunderstandings were cleared up Dr PICKERING admitted that his hypothesis was open to certain criticisms and in so doing indicated that he was prepared to take account of them Perhaps Dr PLATT is right in suggesting that the mathematical method of analysis employed is not entirely satisfactory Moreover Dr PICKERING agreed that a large number of different populations might be involved rather than one homogeneous population

The vexed question of salt the adrenals and hypertension gave rise to an extensive exchange of views There is hardly any disagreement on the whole as to the fact that hyperaldosteronism may possibly accompany essential hypertension But opinions differ concerning the interpretation of this sign and the importance to be attached to it My own feeling is that it is probably a secondary phenomenon and does not indicate the presence of a basic adrenal disorder The same applies to the hypernatriuria occurring in hypertension of every aetiology However the discussion did bring to light various unsuspected correlations between the activity of the adrenals and that of the renin hypertensin system Unfortunately the whole question is complicated by the uncertainties involved in the techniques used I am sure that the specialists will have derived great profit from this colloquium and that they now have a better idea than they did a week ago as to the direction in which to pursue their studies and the snags they must steer clear of

The papers read by Dr BECHGAARD and Dr MILLIEZ gave rise to a most useful discussion on the definition of malignant hypertension It was discovered in fact that there was no absolute clinical criterion of diagnosis and that each speaker had his own ideas on the subject While we all ascribe major importance to the presence of high blood pressure side by side with Grade IV retinopathy some of us pointed out that papilloedema might be absent

in certain patients in whom other signs and symptoms (deterioration in general condition massive albuminuria) indicated a diagnosis of malignancy By contrast Grade IV retinopathy may exceptionally be encountered in patients in whom the blood pressure is only moderately elevated

On the other hand there was unanimous agreement on the necessity of administering vigorous hypotensive treatment in all cases of severe or malignant hypertension although Dr WILSON stressed the shortcomings of such treatment from the theoretical standpoint We were all impressed by Dr SCHROEDER s results which have been largely confirmed by other authors However it appeared that one problem — that of arteriosclerosis — has yet to be solved From the work undertaken by Dr PAGE and his team it is evident that certain patients with malignant hypertension treated with apparent success by means of hypotensive agents succumb to vascular complications of the atheromatous type or subintimal fibroplasia Nor does it seem that hypotensive therapy has any significant influence on the slow progression of benign nephro angiosclerosis

As to the choice of and indications for the various antihypertensive drugs it is generally agreed that the saluretics and guanethidine constitute the major acquisition of recent years The mode of action of chlorothiazide still does not seem to have been definitely elucidated Darenthin (bretylium tosylate) has disappointed most of those who have used it Nevertheless we have good reason to be satisfied with the progress achieved by pharmacologists during the last 10 years That is why I should like to endorse the optimistic views expressed by Dr PLUMMER and to conclude these remarks by quoting his statement to the effect that The hope for further life enhancing drug therapy seems bright indeed

Gentlemen it is time for us to part I sincerely hope we shall have an opportunity of meeting again in the not too distant future and of resuming our discussions where we have now left off

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